HANDBOOK OF ANTIBIOTICS

A. L. BARON

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PREFACE

For some time, the author has felt keenly the need for a compilation of information on the subject of antibiotics. Through the past few years, a veritable flood of technical communications has been pouring forth on this subject, and at this moment the signs point to an increase rather than a diminution in the number of contributions. As a result, the author's experience has been that any attempt to look up any aspect of antibiotics, either from the point of view of determining the overall status of a particular antibiotic or a comparatively minor problem such as the confirmation of a melting point, almost invariably became a major library project. Consequently, in self-defense, and before the amount of material accumulated in the literature became completely overwhelming, the author undertook the task of writing a comprehensive, yet concise, compilation in the form of a handbook or manual of antibiotics.

Acknowledgments are gratefully given to:

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The library of the N. Y. Academy of Medicine; the Chemists' Club Library; the N. Y. Public Library; the Columbia University Library; and the N. Y. Botanical Gardens Library and their staffs, without whose cooperation, the writing of this book would have been well-nigh impossible.

The N. Y. Academy of Sciences for graciously permitting the use of their literature prior to its publication.

A. L. Baron

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CONTENTS

PAGE

PREFACE iii

INTRODUCTION 1

CERTIFICATION OF ANTIBIOTICS BY *Henry Welch* . . . 7

SELECTION AND REGISTRATION OF TRADE-MARKS FOR ANTIBIOTICS BY *Walter J. Derenberg* 13

EXISTING REGISTRATIONS FOR BASIC ANTIBIOTICS BY *Walter J. Derenberg* 29

ACTIDIONE 35

ACTINOMYCES LYSOZYME 37

ACTINOMYCETIN 37

ACTINOMYCIN 38

ACTINORHODINE 41

ACTINORUBIN 41

ALLICIN 43

ALTERNARIC ACID 46

ALVEIN 47

ANEMONIN 48

ANTIMYCIN A 50

ANTI-SMEGMATIS FACTOR 52

ASIATICOSIDE 53

ASPERGILLIC ACID 53

ASPERGILLIN 58

AUREOMYCIN 58

AUXIN 65

AYFIVIN 66

BACILIPIN 68

BACILLIN 69

BACILLOMYCIN 70

BACILYSIN 71

BACITRACIN 72

BIFORMIN 77

BIFORMINIC ACID 78

BIOCERIN 78

BORRELIDIN 79

CANDIDULIN 81

CASSIC ACID 82

Chetomin 83
Chloramphenicol 85
Chlorellin 91
Chlororaphin 92
Circulin 92
Citrinin 94
Clavacin 100
Clitocybin 105
Colicine 106
Colistatin 107
Corticin 108
Crepin 108
Cynematin 110
Dicoumarol 110
Diplococcin 110
Diploicin 111
Endosubtilysin 112
Ei$_5$ 112
Enniatin A 113
Enniatin B 114
Enniatin C 115
Erythrin 116
Eumycin 117
Fumigacin 118
Fumigatin 121
Fuscin 123
Gentisyl Alcohol 124
Geodin 126
Gladiolic Acid 128
Gliotoxin 129
Glutinosin 133
Grisein 134
Griseofulvin 136
Grisic Acid 138
Herquein 138
Hirsutic Acid 139
Inolomin 141
Iodinin 142
Irpexin 143
Javanicin 143
Kojic Acid 145
Lactaroviolin 147
Lateritiin Group 147

LATEROSPORIN 149
LAVENDULIN 150
LECANORIN A 151
LICHENIFORMIN 152
LITMOCIDIN 154
LUPULON 155
MARASMIC ACID 158
MICROCOCCIN 160
MICROMONOSPORIN 161
MUSARIN 162
MYCELIANAMIDE 164
MYCETIN 165
MYCOCIDIN 166
MYCOINES 166
MYCOMYCIN 167
MYCOPHENOLIC ACID 167
MYCOSUBTILIN 169
NEBULARINE 171
NEOMYCIN 171
NISIN 174
NOCARDIN 175
OBTUSIN 176
PENATIN 177
PENICIDIN 181
PENICILLIC ACID 182
PENICILLIN 184
PHENICIN 187
PHTHIOCOL 188
PLEURIN 188
PLEUROTIN 189
PLUMBAGIN 190
POLYMYXIN 191
POLYPEPTIN 199
POLYPORIN 201
PROACTINOMYCIN 202
PRODIGIOSIN 204
PROTOANEMONIN 205
PTERYGOSPERMIN 206
PUBERULIC ACID 207
PUBERULONIC ACID 208
PUCHIIN 209
PYO COMPOUNDS 210
PYOLIPIC ACID 214

QUERCETIN 214
RAPHANIN 215
ROCCELLIC ACID 216
SIMPLEXIN 217
SPINULOSIN 217
STREPTIN 220
STREPTOCIN 220
STREPTOLIN 221
STREPTOMYCIN 222
STREPTOTHRICIN 225
SUBTENOLIN 232
SUBTILIN 234
SUBTILYSIN 239
SULFACTIN 239
TARDIN 241
TERRAMYCIN 242
TOMATINE 247
TRICHOTHECIN 249
TYROTHRICIN 251
USNIC ACID 263
USTIN 265
VIOLACEIN 267
VIRIDIN 268
X45 269
XANTHOMYCIN 270
XG 272
GLOSSARY 275
ANTIBIOTIC-PRODUCING ORGANISMS 281

Synonyms, discarded names and components of multiple antibiotics are not included in the above antibiotics listing. These may be found in the alphabetical order of the ensuing text.

INTRODUCTION

The purpose for which this book was written is the accumulation of published information on antibiotics, not as a miscellaneous collection of facts, as for example by simple abstracts, but organized into a form suitable for ready reference. In keeping with this purpose, all the information in these pages are factual and appear wherever possible in "telegraphic" style, tabulations and statistical summaries. Speculation and theoretical discussions in the literature cited are either held to a minimum or entirely omitted. Needless to say, the author has incorporated no opinions of his own, due not to an exaggerated modesty but to a deliberate desire to produce a work based on facts and data alone, as far as is humanly possible. Parenthetically it may be added that there is nothing more embarrassingly awkward than an outmoded hypothesis or a discarded theory amid an otherwise prosaic factual compilation. To a limited extent, it was found necessary to recalculate or rearrange data, in a very few instances to convert bacterial nomenclature to conform with Bergey, in order to avoid confusion; but as a general rule, the data were compiled in the form appearing in the literature. Where contradiction of fact occurs, both sides are presented.

An earnest attempt was made to avoid the imbalance which frequently results when an author is more interested in some particular phases of his subject and tends to emphasize his special knowledge at the expense of other phases. This tendency was consciously held in check as the material was being compiled and as a result, the author feels that all facets of antibiotics have been covered impartially.

With regard to the type of data compiled, these may be described as primarily "basic" information, *i.e.*, relating more or less directly to the subject of antibiotics themselves rather than their application in applied and theoretical fields. Information which leads too far afield is largely excluded from the text, but is acknowledged by reference to the bibliography. For example, under "Production," the essential steps of the procedure are briefly described, but no attempt is made to enter into the details of equipment, yields, control, etc. This is particularly true of the clinical aspects of the section on "Spectrum in vivo." As compiled in these pages, this consists of tabular data and general conclusions derived directly from the literature. Obviously, a thorough treatment of this material can best be handled by a competent physician, and the reader is referred to the indicated literature on therapy, where such is available.

Primarily, the chief concern of this book is the antibiotic itself as the major source of interest, *i.e.*, its properties, its activity, etc. Of less concern are the associated properties of antibiotics: general problems of antagonism, of therapy, of toxicity, etc. The author will freely admit that he feels as great an interest in a antibiotic which is apparently valueless because of excessive toxicity or other undesirable therapeutic property, as in those which have great potentialities or have actually proved their value in practical application. In some instances, an apparently useless antibiotic is considered of even greater interest because of unusual chemical or biological properties. However, although the author must to some extent plead guilty to a purely academic interest in antibiotics, it should be emphasized that some of the obscure antibiotics mentioned in this volume or others as yet unknown, may suddenly emerge as a valuable product with important practical applications, although not necessarily in the field of therapeutics. Discouraging aspects like high toxicity, etc., may disappear under certain conditions (purification, counter-toxic agents, derivatives, etc.). Thus a serious consideration of all antibiotics without prejudice may prevent the loss of a potentially valuable product. Furthermore, it is conceivable that a particular antibiotic, useless now and in the future from the viewpoint of therapeutic application, may nevertheless be instrumental in clarifying problems of mode of action, of relation between chemical structure and bioactivity and other fundamental problems of antibiotic activity, as well as in nontherapeutic fields, *e.g.*, in industry and agriculture as bactericides and fungicides. In summary, the author wishes to state that he is interested in antibiotics — all of them — regardless of their value at the present moment, or for that matter of their potential value in the future as seen in the present.

Scope and Limitations. The information appearing in these pages covers the scientific literature about two-thirds of the way through 1949, although in a few instances it has been possible to include some antibiotics appearing in print up through the early part of 1950. Apart from the exceptions noted immediately below, all the antibiotics have been covered completely with respect to text and bibliography as regards the original literature. Review and discussion papers are largely omitted. (In one or two instances, the original communication was not available but the title of the reference or its source is included, nevertheless.) Any omissions other than those noted are to be considered as oversights, of which the author would like to be informed and for which he offers his apologies and the promise that they will be included in a subsequent edition.

The following substances, some of which may be regarded as antibiotics, were omitted from the text for the reasons mentioned:

(1) *Unnamed antibiotics.* These are omitted from purely practical considerations. At first thought, this may appear to be an apparently arbi-

trary decision, but careful consideration will make it clear that no other recourse is possible. In the first place, there have been described in the literature a large number of "active" extracts which vary in purity from crude extracts to pure crystalline products. Obviously, it would be confusing to include all these on an equal basis; yet it would be impossible to draw the line without actually becoming arbitrary. Secondly, these are identified only by the taxonomic designation of the producing organism, *i.e.*, as "Factor from," "Active principle from," etc. To include terminology of this type among the relatively precise procedure of simple names is not only awkward, to begin with, but will become overwhelmingly complicated in the future as additional information appears in the literature. This may be illustrated by imagining the resultant confusion in nomenclature should, for example, "Factor from *A. niger*" be reported also to be derived from *P. notatum;* or if *A. niger* be found to produce two or more separate factors! Obviously, this situation would be saved if arbitrary names were assigned. The identity of two or more differently named antibiotics creates much less confusion than would be imagined. When this occurs, the prior name, by date, is given preference, and the others merely listed as synonyms, remaining as points of reference.

(This criterion is one that applies only for the purposes of this type of compilation, and should not be interpreted as a criticism of the excellent studies which have been carried out in methodically going through a series of genera and species for indication of antibiotic production. As a matter of fact, the author has a very high opinion of these inclusive studies, particularly as source material in the search for new antibiotics, and has seriously considered the possibility of assembling this type of information, either in a separate volume or as a part of a future edition of this book. On the other hand, the author does not wish to be interpreted as lending support to the indiscriminate naming of "new" antibiotics without regard to their possible identity with those already isolated. It should be taken for granted that any announcement of a "new" antibiotic has been preceded by sufficiently exhaustive biological and chemical tests to insure that it is indeed "new.")

(2) *Substances primarily lytic in properties, e.g.*, enzymatic or surface-active, and have been studied primarily from the viewpoint of the lysis of dead bacteria. Where some activity against living bacteria has been indicated, they are included and described briefly.

(3) *Antibiotics which are ill-defined, questionable or have obviously exaggerated properties.*

(4) *Antibiotics having obviously feeble antibiotic properties.* In borderline cases, however, these are given the benefit of the doubt and are included.

(5) *Lysozymes, antibodies, and certain well-known metabolities.*

Penicillin and Streptomycin. Although all other antibiotics are described completely, it was found necessary for the purposes of this book to cover these two antibiotics in an extremely abbreviated form, without references other than those books which have large sections on penicillin and on streptomycin. An exhaustive treatment would completely unbalance this book by virtue of the bulk added and devoted to penicillin and streptomycin. (The complete bibliography of these, if included, would require more than the number of pages in this entire volume, exclusive of the space required for the text.) As a result, the newer and lesser-known antibiotics would be overshadowed by the mass of material devoted to two well-known antibiotics, and the use of the book for general reference to antibiotics would become more difficult. A compromise solution to this problem was given momentary consideration, *i.e.*, a "selected" bibliography and a curtailed version in place of a complete text on penicillin and streptomycin, suited in bulk to the complete coverage of the other antibiotics. This compromise solution was never given serious thought, however. The author felt at the time, and still feels, that if the coverage could not be complete, it had better not be included at all — that an incomplete bibliography and text is neither fish nor fowl. An additional factor in confirming this viewpoint has been the appearance of several excellent books on penicillin and streptomycin. No useful purpose would be served by rehashing material which has been covered in these texts. As a result of all these considerations, penicillin and streptomycin were written in extremely summary form, with facts and conclusions boiled down to the least common denominator and a bibliography consisting only of the aforementioned books. This summary coverage is primarily for the purpose of "rounding-out" the antibiotic listing and to avoid the obvious gap that would exist if these two were omitted entirely. Serious thought has been given to working up a companion volume to the present one, to include only penicillin and streptomycin.

DEFINITION OF AN ANTIBIOTIC

In the most parsimonious sense, an antibiotic is merely a member of the group of substances generally referred to as antiseptics, disinfectants, bactericides, fungicides, etc. There is little or no relation, chemically, biologically or otherwise, between all the various antibiotics, other than their ability to affect adversely the life processes of certain selected microorganisms. Despite the absurdly simple character of antibiotics there is a tendency on the part of a few observers in this field to assign them exaggerated attributes, which add nothing to their definition and to some extent even border on the mystic. Needless to say, this type of thinking will be ignored in this work. With regard to the function of antibiotics in contributing to the survival value of the antibiotic-producers, little or no evidence in favor of this viewpoint has been uncovered, and in any case it is outside the province of a book of this type.

As defined by Waksman [*Science*, **110,** 27 (1949)], "An antibiotic or an antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms." This definition states that an antibiotic must be a product exclusively of microorganisms, from which it may be inferred that antibiotic-like substances produced by macroorganisms are not to be considered antibiotics but must be given other designations. On the other hand Benedict and Langlyke [*Ann. Rev. Microbiol.*, **193** (1947)] define an antibiotic as ". . . a chemical compound derived from or produced by living organisms, which is capable, in small concentration, of inhibiting the life processes of microorganisms." It will be noted in this definition that the qualification requiring that the antibiotic be produced by microorganisms only, is omitted and another qualification added, to wit, small effective concentration. The author is inclined to concur with the definition of Benedict and Langlyke. (However, it should be pointed out that these two authors disqualify penatin as an antibiotic, despite the fact that it fits all the conditions of their definition, on the grounds that penatin acts by producing hydrogen peroxide through enzyme action, and that it is the hydrogen peroxide which is the bactericidal agent. This conclusion implies that an enzyme cannot be an antibiotic, and may mean, with regard to the mechanism of action of antibiotics, that once the mechanism of action of an antibiotic is completely explained, it will cease to be regarded as an antibiotic. To this, the author cannot agree.)

The adequacies and inadequacies of the above definitions and of many others which have appeared in the literature are source material for endless discussion. However, as far as the purposes of this book are concerned, the author has cut the Gordian knot by proposing no new definition at all. Instead, an antibiotic is considered as such if it satisfies, more or less, all of the following conditions:

(1) It is a product of metabolism (although it may be duplicated or even have been anticipitated by chemical synthesis).

(2) It antagonises the growth and/or survival of one or more species of microorganisms.

(3) It is effective in low concentration.

A concomitant low toxicity to higher plants and animals at therapeutic levels is a desirable feature, but not a prerequisite.

It may be emphasized that the above criteria are not to be interpreted rigidly and are used only for convenience to fit the chemically and biologically miscellaneous compounds covered in these pages. In practice, an antibiotic will be accepted as such when so considered by reliable observers and in cases of reasonable doubt.

The author feels strongly that any distinction between antibiotics on the basis of their derivation from micro- or macroorganisms is artificial and may lead to confusion in the future as newer antibiotics increase in number. To restrict the term *antibiotic* to metabolic products of microorganisms alone would be undesirable for the following reasons:

(a) By inference, it excludes potentially the valuable antibiotic substances of higher plants and animals from future investigation.

(b) By inference, it arbitrarily assigns unique and unusual qualities to the metabolism of all microorganisms as contrasted to all macroorganisms. Apart from other considerations, actually there are no chemical or biological criteria for differentiating the antibiotics from microorganisms from those of macroorganisms.

(c) It needlessly brings up the necessity for coining new names for the antibiotic products of macroorganisms.

(d) It will introduce unnecessary confusion when specific antibiotics are found to be produced by both micro- and macroorganisms. Thus, citrinin, an antibiotic derived from *Penicillium citrinum* and lower fungi, has been reported as occurring in large quantities in the leaves of *Crotolaria crispata,* a flowering plant of tropical North Australia. Obviously it would be illogical to classify citrinin from *Penicillium citrinum* as an antibiotic, and the chemically identical substance from *Crotolaria crispata* as a nonantibiotic. Similarly, it seems unreasonable to accept phthiocol, derived from *Mycobacterium tuberculosis,* as an antibiotic and yet to reject plumbagin, derived from the higher plant *Plumbago europaea,* when their chemical structure indicates that one is an isomer of the other.

Consequently, with the above justification, the substances covered in the ensuing pages are considered antibiotics on an equal basis, regardless of the genus and species of the producing organism.

CERTIFICATION OF ANTIBIOTICS

HENRY WELCH

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No other class of drugs has had a more profound effect on public health than the antibiotics. These products of the metabolism of microorganisms have provided medical science with extremely effective weapons for combating a long list of serious and crippling diseases including those of bacterial, virus, and rickettsial origin. Because of their continuing importance and widening use by the medical profession, more of each is manufactured and used every month. Only eight years ago, in 1942, we had insufficient penicillin to treat a single case; by 1944 we were supplying not only our own armed forces but those of our allies as well; and by January, 1949, over eight trillion units of penicillin were being produced monthly. One year later, in January, 1950, production had again doubled to the enormous figure of sixteen trillion units per month. Because of the "know-how" gained with penicillin, production of streptomycin increased in a similar rapid manner, and in January, 1950, six American manufacturers were producing this drug at the rate of eight million grams per month. The quantities of aureomycin, chloramphenicol, and bacitracin produced are also increasing at a tremendous rate. Since these drugs are produced by individual manufacturers, actual production figures are not available, but it can be said that the quantities manufactured of each are increasing rapidly. This is worthy of note when one considers that all of these drugs are similar in their effects on certain diseases.

Penicillin was a war drug, and its development came in a period of history when there was a great demand for drugs useful for treatment of war casualties. Although it was discovered by Fleming in 1929, nothing was done with penicillin until 1938 to 1941, when the Floreys, Raistrick, and others became interested in its possibilities as a means of combating infections in the war-wounded. The drug was extremely unstable, and it was through the early work of these English investigators that this problem was partially answered. Since England was unable to establish manufacturing facilities for penicillin because of the war, Florey came to this country to stimulate the interest of the United States pharmaceutical industry. Through the cooperation of the National Research Council, the medical profession, the War Production Board, the Food and Drug Administration, and industry, specifications were set up for penicillin, and funds were made available where necessary by the Government to initiate production of the drug. During the early developmental stages there were many uncertainties in the production of this fermentation product and rigid control was a necessity. Within the plants, production control was difficult to establish and maintain; stability was a major problem, and the insurance of sterility and freedom from pyrogens and toxic substances required special attention. In addition, relatively early in the history of the drug it was recognized that there were several "penicillins" with different potencies, which complicated the picture that much more. Because of the need, the armed services obtained practically all the penicillin in the first three years of production. Recognizing the difficulties of production and assay of this important drug, the armed services requested the Food and Drug Administration to set up specifications for penicillin and to check each batch manufactured against those specifications. The Food and Drug Administration established laboratories for this purpose during 1942 and 1943, and these were the forerunners of the present pretesting program for antibiotics.

By early 1945 it was obvious that supplies of penicillin would soon suffice for civilian as well as military demands. This raised anew the question of appropriate control measures, since the procedures applying to control of penicillin for the armed services would not apply to penicillin intended for civilian use. Following a series of conferences between Government and industry, it was agreed that a system of predistribution testing of each batch by the Food and Drug Administration would be in the public interest. The Food, Drug, and Cosmetic Act of 1938 contained no provision which would cover such predistribution testing, and it was therefore necessary to request Congress to amend the existing law to provide for a system of certification for penicillin. The amendment was passed on July 6, 1945. It directed the Administrator of the Federal Security Agency to provide for the certification of drugs composed wholly or partly of any kind of penicillin. It authorized him to set up such standards of identity, strength, quality, and purity as he found necessary to adequately insure safety and efficacy of use. It banned interstate traffic in any penicillin-containing drug unless it was from a batch that had been certified.

Certification of penicillin, which in effect means examination by the United States Food and Drug Administration of each batch of the drug produced for identity, strength, quality, and purity before it is shipped in interstate commerce, was not initiated with the antibiotics, but originated over forty years ago with the voluntary certification of coal-tar food colors. Certification of these coal-tar colors became mandatory in 1938, and this was followed in 1941 by the certification of insulin. The certification of penicillin established a precedent as far as the antibiotics were concerned, and no opposition was offered by industry to the inclusion of streptomycin under a certification system by an amendment made March 10, 1947. Two years later on July 13, 1949, the law was again amended to include the antibiotics aureomycin, bacitracin, and chloramphenicol.

The actual scope of antibiotics certification is one that deserves special consideration. Certification is neither necessary nor practicable for drugs generally. But the important antibiotics possess peculiar and unusual characteristics that make certification a useful and valuable procedure in safeguarding the public health.

These characteristics which are found in one or more of the antibiotics are:

A. Demonstrated therapeutic value in one or more disease conditions which constitute a major public health problem;
B. Probability of contamination with undesirable materials unless manufactured under the most careful controls;
C. Inherent instability, sometimes resulting in decomposition products which may cause untoward reaction;
D. Marked variation in therapeutic response with changes in vehicles or routes of administration;
E. Necessity for evaluation by biological or other methods lacking in precision.

The primary criteria for applying the certification procedure are the importance of the drug in combating serious and crippling diseases and the difficulty in evaluating the final commercial material. Certification does not relieve the producer of his responsibilities, but forcefully emphasizes the vagaries of the drug and the need for a stringent internal control system.

The establishment of regulations under the antibiotics amendment follows, at the present time, a fairly well-established pattern. Monographs are prepared establishing standards of identity, strength, quality, and purity for each dosage form, and a companion monograph is included to cover the tests and methods applied to determine conformance with the standards. When a new product containing a certifiable antibiotic is developed, the producer requests certification of the new pharmaceutical and furnishes the Commissioner of Food and Drugs with a statement of the conditions for which the drug is to be used; a full report of the investigations conducted to show the drug is safe and efficacious for such conditions; a full list of the articles used as components of the drug and the quantity of each; a description of the methods he proposes to use in the manufacture, processing, packaging, and control of the drug; a description of methods that can be used in testing the new product; samples of the drug and its components, and specimens of the contemplated labeling. If the information submitted is satisfactory to the Commissioner he then recommends to the Administrator of the Federal Security Agency that regulations be issued by publication in the Federal Register to provide for the certification of the proposed drug.

Once a monograph has been established, the manufacturer, or any other who has the legal right and meets the requirements of the regulations, may submit batches of the drug for certification. When samples have been received they are subjected to the tests described in the Official Tests and Methods, and if they meet the requirements, the batch is certified for distribution. If the batch does not comply with the standards, certification is refused and the batch must either be reworked or destroyed. It cannot be distributed.

The tests performed on samples of penicillin, streptomycin, dihydrostreptomycin, aureomycin, bacitracin, and chloramphenicol are completed and the results reported to the manufacturer within a four to five day period. By arrangement with the Administration many manufacturers obtain certificate numbers by phone or wire on the day the tests are completed. In any case, each manufacturer receives a formal document of certification on each batch of drug tested if it meets the standards of identity, strength, quality, and purity set up by the regulations. At the present time there are thirteen basic manufacturers of penicillin, seven of streptomycin or dihydrostreptomycin, one of aureomycin, one of bacitracin, and one of chloramphenicol.

Except for aureomycin and chloramphenicol, which are owned and produced by only one manufacturer each, a number of manufacturers purchase antibiotics for manufacture into a variety of preparations. These manufacturers usually purchase certified material for this purpose. However, a manufacturer may purchase uncertified material either for manufacturing use or for repacking. Material so purchased is subject to exemptions which allow the basic manufacturer of the drug to ship in interstate commerce without certification to repackers or to manufacturers of preparations. If a manufacturer of a preparation uses an antibiotic not previously tested by the Food and Drug Administration, he must submit for assay samples of the antibiotic used, as well as samples of the preparation. The preparation must be certified in any event, whether the antibiotic used in its manufacture was certified or not. This is true also of repacked antibiotics.

During the early days of penicillin production, very little of this drug was shipped in export. As time has gone on, however, there has been an increased amount exported. In the case of streptomycin, the reverse of this was true in that large quantities of streptomycin were exported during the early days of production, although the amount exported has been reduced to some extent during the past few years. Antibiotics that are shipped in export do not require certification unless the country to which the product is going requires it. Section 801(d) of the Federal Food, Drug, and Cosmetic Act provides an exemption if the drug is plainly marked for export if it meets the specifications of the foreign buyer and if it complies with the regulations of the country to which it is being shipped. Actually most of the antibiotics shipped in export are certified or comply with the standards prescribed for certification.

Up to the present time, practically no antibiotics have been imported into the United States. With the increased production abroad, however, it is not unlikely that eventually penicillin and perhaps streptomycin may be imported. If they are, they must meet the same requirements as apply to the domestic products.

When the penicillin amendment was enacted it was recognized that this type of control should continue only so long as it was necessary to insure safety and efficacy of use. The law directed the Administrator, whenever in his judgment the certification requirements with respect to any drug are not necessary to insure safety and efficacy of use, to promulgate regulations exempting the drug from such requirements. It was under this provision that both crystalline sodium and crystalline potassium penicillin G were released from the certification requirements on April 1, 1950.

SELECTION AND REGISTRATION OF TRADE-MARKS FOR ANTIBIOTICS

WALTER J. DERENBERG *

Trade-Mark Counsel, U. S. Patent Office

Introduction

It may seem strange to discuss matters of legal protection and the selection of trade-marks within the framework of a scientific book which presents in alphabetical order the composition and characteristics of hundreds of known antibiotics under their scientific and generic names without any reference to the manner in which these products or their derivatives or by-products may be treated commercially. Hence, it must be stated at the outset that the following brief discussion of trade-marks on antibiotic products will not deal with any of the scientific names which form the basis of this Handbook, but will treat only some problems arising in connection with the selection and protection of commercial names which a manufacturer of a basic or secondary antibiotic product may desire to develop from a business point of view.

Any such discussion involves two basic distinctions. First, with regard to the products themselves, a distinction is drawn for practical purposes between so-called basic antibiotics now commercially available, of which there are not more than seven † at the present time, and derivatives from these basic antibiotics, of which there are an ever-increasing number sold under a great variety of trade-marks. Such registrations of trade-marks for derived or composite antibiotic products are, for instance, "PAR-PEN" (registered by Smith, Kline & French Laboratories for an "Antibiotic Mixture for topical use"); "SOLUTHRICIN" (registered by Sharp & Dohme, Incorporated, for an "Antibacterial Preparation"); "TERSAVIN" (registered by Hoffmann-La Roche Inc., for a "Medical Preparation with Vasoconstrictor and Decongestant Action for Use in the Treatment of Sinusitis and Related Conditions"); "PENI-CRYSTIN" (registered by The Wm. S. Merrell Company, for an "Antibiotic Product in Crystalline Form"), and many others. In view of the enormous growth of the number and variety of products of this kind, it is hoped that this brief chapter, discussing certain basic legal principles, may be of some small assistance to manufacturers of such products in the selection of a commercial name therefor.

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* The views expressed in this article represent entirely the author's personal views, and do not in any way reflect the views of the United States Patent Office.

The author wants to take this opportunity to express his thanks to Dr. Henry Welch, Director, Division of Antibiotics, Federal Security Agency, and to Dr. Karl Bambach, Assistant Vice President of the American Drug Manufacturers Association, for their suggestions and assistance in the preparation of this article.

† Penicillin, Streptomycin, Chloramphenicol, Aureomycin, Bacitracin, Tyrothricin, and Terramycin.

Secondly, an important distinction must be made, particularly with regard to commercial names of basic antibiotics, along the lines of certain medical and pharmaceutical considerations and policies on the one hand and strictly legal problems on the other. It then becomes immediately apparent that inventors and manufacturers of new basic antibiotics are faced with certain unique problems which rarely confront the manufacturer of products in other branches of business or industry. These unique problems are not so much concerned with the usual question of what commercial name to select and how to protect it, as with the more fundamental issue of the advisability of selecting any legally protectable name at all, in addition to the product's scientific name and the name of the manufacturer. Any discussion of the legal prerequisites for registration of antibiotic names as trade-marks must, therefore, necessarily be preceded by a brief analysis of the position of the Council on Pharmacy and Chemistry of the American Medical Association, and the medical profession in general, with regard to the naming of new antibiotic products.

I. Selection of "Product" and "Protected" Names Under the Rules of the Council of Pharmacy and Chemistry

In the terminology of the Council, trade-marks for new drug products are customarily referred to as "protected names." Neither the Food and Drug Administration nor the Council actively promotes the use of such names. On the contrary, with regard to many new drug products, and specifically antibiotics, there is a tendency to discourage the use of trade-marks. The Council has always followed the theory that from the viewpoint of the public and the medical profession, it would be more advantageous to concentrate upon use of a single generic name for such drugs without burdening the memory of the physician or the public with hundreds of different trade-marks. As a result of this attitude, an editorial was published in the *Journal of the American Medical Association* (February 2, 1946) under the title "Silly Names for Penicillin Products," ridiculing the adoption of so many protected names for these products. The editorial said of the antibiotic manufacturers that: "Instead of selling penicillin under its simple and well known name, they have become downright silly and offer penicillin under so many names that only a professional solver of cross-word puzzles could guess the nature of the products that these names conceal." A later editorial which appeared in the issue of July 17, 1948, said: "In a previous editorial attention was called to the combination of the manufacturer's name with 'cillin.' Since there were almost thirty manufacturers, that offered almost thirty possibilities. Now the emphasis seems to be inclining toward the port of entry plus 'cillin.' The Council on Pharmacy and Chemistry in recent years has tried to be more than reasonable about the acceptance of fanciful names for widely used products of varying manufacture. Even the patience of the proverbial Job would be tried by contemplation of a list such as is here cited."

The view of the Council that the use of the basic generic term of an antibiotic should be promoted rather than the added use of certain "protected" names is reflected in the Council's rules on "Nomenclature" and "Trade-Marks." * Apart from the basic rule that no "protected" name should suggest a therapeutic use of the product, but should indicate the "potent element or constituent" of the product — although, if it did only this, such "protected" name would not be registrable under the Federal Trade-Mark Act (see below, II) — the policy of the Council at present is stated to be as follows:

"The Council recognizes the importance to a manufacturer of a name which shall identify his own product. In the case of new discoveries, it recognizes the preferential right of the discoverer to choose a name. It has become the custom to use such names in connection with a properly scientific, systematic common or generic designation. The Council requires that such a generic name appear on the labels of accepted preparations marketed under trademarked names and that it be given at least half the display value of the protected name."

It should also be noted that "protected" names are not accepted for U.S.P. or N.F. articles or for nonofficial modifications or dosage forms of such articles unless such names were in commercial use before the products became official, or unless they concern products which are otherwise entitled to protected names in the judgment of the Council. It is interesting to observe that the Council rules requiring the giving of at least half the display value to the product name, as is afforded the "protected" name, do not go quite as far as the provisions of the Public Health Service Act of 1944, which, in Section 351, provide that in case of serums, toxins, viruses, and similar products, the generic product name must be given "precedence in position" and "precedence in prominence" over any trade-mark or trade

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* It is interesting to note that, in a paper before the Pharmacopoeial Convention held at Washington, D.C., May 9–10, 1950, Dr. John C. Krantz of the University of Maryland School of Medicine made the suggestion that, contrary to the rules of the Council, trade-marks and commercial names of drugs should be given preference in the "U. S. Pharmacopoeia," because such names would become more readily and widely known among the profession than the scientific names of the drugs. A summary of Dr. Krantz' interesting paper may be found in *Drug Trade News* (May 15, 1950).

name used. The requirements of the Council, on the other hand, are satisfied if the common or generic name is not "unduly subordinated" to the trade-mark in the labeling and advertising.

In order to avoid confusion or criticism, the Council not only is willing, but deems it highly desirable, that trade names intended to be used should be submitted to it before they are publicized. In other words, in addition to having a preliminary search made through Patent Office records or through the files of the combined Bureau of the A.D.M.A. and A.P.M.A., a proposed trade-mark, to be used on an antibiotic or derivative thereof, may, under the Council rules, be submitted to it for informal acceptance before the drug itself is commercially introduced on the market.

Another restriction concerning the use of trade-marks is found in Rule 5 of the Rules, under which the Council insists that coined names for salts should indicate the components of such salts. In this respect, the Council points out:

"Difficulty frequently arises from the application of coined names to salts. For example, a firm introduces the hydrochloride of a synthetic base under the name 'Artificialine.' Subsequently the firm decides to introduce the lactate of the same base. If this is called 'Artificialine lactate' the name 'Artificialine' will now mean the base instead of the hydrochloride which is being marketed under that name. In order to avoid this confusion the Council holds that coined names for salts will not be accepted unless such names indicate the components of such salts, thus 'Artificialine hydrochloride'; the name 'Artificialine,' unqualified, is acceptable only for the base."

On the other hand, the Council has no objection to emphasizing the protected nature of the trade-mark by either putting the mark in quotation marks or adding an asterisk to it, or otherwise indicating the fact that it is a trade-mark. A similar rule with regard to the use of coined names for new substances provides that as far as the Council is concerned, they will be accepted only if such names indicate the type or dosage form of the preparation, "thus, 'Elixir of Aliphal,' 'Aliphal Powder,' not 'Aliphal' unqualified."

Furthermore, the Council rules contain important restrictions with regard to the use of numbers and letters in names of new drugs. Under the Trade-Mark Law, such letters and numbers may be registered as trademarks unless they are actually descriptive, but the Council objects to the use of such devices as part of protected names on much broader grounds. It says, in this regard:

"Since the use of numeral or alphabetical designations in connection with drug names tends to take the emphasis away from the name and to displace the name, thus leading to confusion, the Council will not recognize the name of a drug in which the numeral or letter is an integral part of the name, except in special cases where the use of a numeral or letter seems desirable because further improvement of the product is anticipated, in which case the Council may grant a special exemption from the rule. Under this rule the use of numerals or letters in connection with the name of a product will not be permitted on labels or in advertising, unless the numeral or letter is clearly separated from and subordinated to the name by type and if feasible by position. This rule does not apply to price lists and catalogs."

While the Council is thus much less concerned with the development and protection of trade-marks than with the establishing of proper scientific names and the greatest possible degree of uniformity in the use of such names for basic products by the medical profession, it will permit, as indicated, not only the use of newly coined words as trade-marks in conjunction with the scientific name, but it also recognizes and, indeed, to some degree encourages the use of so-called "house marks" or, as the Council calls them, "line names." Thus the Report of the Council for 1949 states:

"The Council also recognizes 'line' names, when not in themselves misleading and when not used as the name for a single product. The firm's name or initials, when not of such character as to be misleading or cause confusion, may also be used thus."

In other words, from the Council's point of view, it would seem much preferable to emphasize the name of the manufacturer as a trade-mark on all products made by him, including particularly new antibiotics, such as, for instance, "Penicillin Schenley," than to add a third newly coined word to the scientific name and to the name of the manufacturer. While, as will be pointed out below, the name of the manufacturer alone, if it consists merely of a common surname, cannot be registered as a trade-mark without having acquired "distinctiveness" in trade, most drug manufacturers' names can easily satisfy this requirement of distinctiveness so that such famous house marks as, for instance, Pfizer, Merck, Roche, and Parke, Davis, would all be clearly eligible for trade-mark registration today. It should, however, be mentioned that the law — contrary to the Rules of the Council — makes a distinction between a trade-mark as such and a mere "trade name." Mere trade names, as such, are not registrable as trade-marks. This means in actual practice that if registration of a name such as Pfizer or Merck is sought and the specimen submitted to the Patent Office shows nothing but a statement, "Manufactured and Distributed by —— Company of New York, New York," such use would not be held trade-mark use, but only use of the business name and address. For that reason, it is advisable to use an abbreviated version of a well-known manufacturer's name as a trade-mark, or to put the entire name without the address and location into some more or less distinctive design, such as a diamond or triangle, in order to emphasize the trade-mark character of the word. If this small precaution is taken, there should never be any difficulty in getting legal protection and registration for so-called house marks of well-known drug manufacturers.

In naming a new antibiotic, one difficulty will arise which is not usually found in the case of other new drugs. Normally, the chemical composition of a new drug is known and the new generic or scientific name of the drug will be such chemical name or, if too complicated, an abbreviated version thereof. In the case of new antibiotics, however, the chemical composition may not be known, and hence the scientific name to be given to the product has to be coined. Under such circumstances, a manufacturer, if he wants to give his product a "protected" name, would have to resort to the device of suggesting *two* newly coined words, one to be used as the scientific name for the antibiotic, such as most of the names discussed and analyzed in the main portion of this book, and, in addition, a coined word which would be his particular brand of the new product. Or, if in accordance with the general approach of the Council and many members of the medical and pharmaceutical professions, the manufacturer should not consider it good policy to introduce a product under two newly coined names, one of which would be a "protected" name, he might then confine himself to the use of the new generic name in addition to his own house mark. In the rare case in which the chemical composition is known and definable, it may be more practical for its discoverer immediately to adopt a protected name in addition to the name indicating its chemical composition. This was recently done by Parke, Davis & Company in connection with a new antibiotic whose chemical composition was known. Their patent is described as a "process for obtaining Chloramphenicol," a name which Parke, Davis & Company gave to this new chemical compound and derived from its chemical ingredients. At the same time, Parke, Davis & Company began to advertise the product under the trade-mark "Chloromycetin" and sought and obtained registration of the word in the Patent Office. Having thus secured registration and legal protection of the trade-mark "Chloromycetin," it is now the manufacturer's task to protect this name against any generic use by others, either commercially or in scientific literature.

II. Registration of Antibiotic Trade-Marks in the U. S. Patent Office

It is, of course, clear that neither the acceptance of a protected name by the Council nor registration of such name with the combined Trade-Mark Bureau of the A.D.M.A. and A.P.M.A. provides any assurance that the mark selected will qualify for registration as a trade-mark under the Federal Trade-Mark Act of 1946. In order to be registrable under that act, the mark must not run afoul of certain statutory prohibitions, of which the following are particularly important here:

(1) The mark must not consist of or comprise any immoral, deceptive, or scandalous matter, or matter which may falsely suggest a connection with living or dead persons, institutions, or national symbols. Thus, no mark containing the Red Cross symbol can now be registered unless it had been in actual commercial use prior to the year 1905.

(2) A mark which, when applied to the goods, is merely descriptive or deceptively misdescriptive is not registrable. Under this section, no generic or common descriptive name of a drug product can ever qualify for registration, and it may not always be easy to select a name which satisfies the Council's preference for a name indicating "the potent element, or constituent" product on the one hand, and the legal requirement for a non-descriptive name on the other.

(3) A name which, when applied to the goods, is primarily geographically descriptive or deceptively misdescriptive is not registrable; (but see the exception noted under (5)).

(4) A name which is "primarily merely a surname" does not qualify, unless it falls within the exception noted under (5). Under the statute, names such as Pfizer, Merck, Lederle, Schenley, etc. are not registrable trade-marks *per se* unless they come within the rule hereinafter set forth. Under the present law, not even combinations of two or more surnames, such as Parke, Davis; Hoffmann-La Roche, and others, are acceptable if the prerequisites mentioned under (5) are lacking. Until the new Trade-Mark Act of 1946 became effective, surnames could be registered if they were "distinctively displayed," but this was held to refer to a person's signature only and not to cover other forms of distinctive display. The new Act of 1946 does not embody the distinctive display exception.

The language of the new act, "primarily merely a surname," has been recently construed by the Patent Office to permit registration of the combined Christian and surname of a person. Thus, under the present practice, a name such as Eli Lilly, since it indicates the complete name of a person, could be registered, even if the surname were as common a surname as Smith or Davis. It should be noted, however, that if the name involved is that of a particular living individual, the new Trade-Mark Act requires such individual's consent in writing before his name or portrait can be used as a trade-mark.

(5) Before 1946, such famous names as, for instance, Merck, Upjohn, Ford, Waterman, etc. were registrable only in one of three cases:

(a) If they had been used exclusively as trade-marks for ten years prior to 1905 (the so-called 10-year proviso). Very few now famous names would qualify under that proviso.

(b) If they were "distinctively displayed" in the form of a person's signature.

(c) If the application were filed under the Supplemental Trade-Mark Act of 1920, which afforded practically no domestic protection, but served only as a basis for registration in other countries.

Under the Trade-Mark Act of 1946 it is provided that any surname or geographical or descriptive term may be registered and entitled to full protection under the statute, despite its original weakness, if a showing can be made that such marks have become "distinctive" in commerce. Five years of substantially exclusive and continuous use of the mark before the filing date of the application may be accepted by the Patent Office as *prima facie* evidence of such distinctiveness, or, as the courts have called it, "secondary meaning." However, an applicant need not rely on such five years' use if a showing can be made that an originally ineligible mark has actually acquired the status of a distinctive trade-mark before the end of the 5-year period. It is one of the significant advantages of the new Trade-Mark Act that all such famous names as, for instance, Abbott, Sharp & Dohme, and Parke, Davis, are now registrable on the Principal Register upon a satisfactory showing of acquired distinctiveness.

As indicated, even a descriptive term may acquire distinctiveness so as to become a full-fledged trade-mark under the new act. This would apply, for instance, to such well-known marks as "DYANSHINE" for a shoe polish, or "L'ORIGAN" for a perfume, but — and this cannot be overemphasized — it will never apply to a name which is not only descriptive of the appearance, ingredients, or characteristics of an article, but which is actually a *generic* or common descriptive term in the sense that it is the accepted general designation for the article itself. Thus, it was recently held that the words "SPAR Composition" for a varnish were "generic" as distinguished from descriptive, and therefore incapable of acquiring distinctiveness as a trade-mark. Similarly, it was held that even under the new act, the words "FLEUR DE SOUFRE" were generic and incapable of acquiring distinctiveness for a sulphur product. Therefore, the majority of the product names hereinafter discussed are incapable *ab initio* of acquiring trade-mark significance because they are considered in legal contemplation to be generic names, as distinguished from merely descriptive names. Once the name of an antibiotic or any other product has actually become generic in this sense, it ceases to be a registrable trade-mark and, if actually registered, may become subject to cancellation. It would therefore be quite risky for the discoverer of a new antibiotic to give it only one name and at the same time seek to protect such name as a trade-mark. What happens in such cases is well illustrated in a leading case in which a new natural substance was discovered and the name "Kelp Ore" given to such new product. The name was registered at the United States Patent Office, but when the owner thereof subsequently sought to restrain others from calling the product "Kelp Ore," he lost out on the ground that the words "Kelp

Ore," even though registered, were in fact a generic name for the new substance rather than a trade-mark. The court there said:

"Hence, if we assume plaintiff's good faith, it must be held that, in calling the newly discovered material 'Kelp Ore' and making it generally known as such during the period of 17 years, he intended the name to be generically descriptive, and he cannot now adopt it as a trade-mark, to the exclusion of the right of others to apply the name to substances of similar origin and character."

The general rule which prevails today is aptly stated in the *Restatement of The Law of Torts*, as follows:

"When an article is newly discovered and is patented, or its composition is kept secret, the person who exploits the article in commerce may give it a name which is the descriptive or generic designation of that type of article. *Such a name is not a trade-mark and use of it as such does not make it a trade-mark either during the life of the patent or secret or thereafter * * *. But if, in addition to such name, he adopts a designation to distinguish the article of that type with which he is associated, that designation may be a trade-mark during the life of the patent or secret and continue to a trade-mark thereafter.*"

This author knows of no better yardstick for antibiotic manufacturers, in the selection of names for new products.

(6) Even if the name of a new product does not run counter to any of the foregoing prohibitions, there are still two other hurdles which must be cleared before the mark will finally enjoy the benefits of the registration statute.

(a) The Patent Office must satisfy itself that the mark does not so resemble another mark previously registered or in use as to be likely to cause confusion or mistake or to deceive purchasers. In other words, the Examining Corps of the Patent Office will make an exhaustive search of pre-existing registrations and if one be found which is either identical with or closely similar to the applicant's mark and used on identical or similar products, the application will be rejected on this ground. Under the new Act of 1946, it is no longer required that in order to be thus rejected, the products must have "the same descriptive properties." It is sufficient if they are so closely related as to create an impression in the public mind that there may be some connection in trade between the two products or their manufacturers.

(b) If the Patent Office is satisfied that the mark applied for does not conflict with a previous registration, the mark is then published in the Official Gazette for opposition. An opportunity is thus afforded to competitors or other interested parties to allege that they would be damaged if the applicant's mark were registered. Such damage in case of a new drug product might exist in one of the following cases:

(1) A conflict with an opposer's previously registered mark, which the Patent Office did not consider sufficiently close to create a likelihood of confusion; or
(2) In the opposer's prior use of an unregistered trade-mark or name (including for this purpose, adoption by a newcomer of part of an opposer's corporate name as a trade-mark); or
(3) Where an allegation is made that the mark sought to be registered is in fact a descriptive or generic term which is so used by opposer and other members of the industry.

If an opposition is sustained on any of these grounds, the mark will not be finally registered, even though it had already been published in the Official Gazette. It may be noted that, particularly in connection with drug products, the attention of the Patent Office is occasionally called by other Government agencies or scientific organizations to the fact that certain marks published for opposition are in fact generic terms, and are so considered and treated in scientific literature. In such instances, it is the policy of the Patent Office to withdraw such marks from publication and to reconsider their registrability *ex parte;* that is, without the necessity of an opposition proceeding.

In determining whether the name sought to be registered for a new drug product may or may not be confusingly similar to a pre-existing mark, it is, of course, impossible to lay down any hard and fast rules, but certain general principles have been developed in recent years which may be of some assistance in guiding drug manufacturers in this respect. After having had a search made through the records of the Patent Office — which, since a recent important change in Office rules, may now include a search of pending applications — the following general principles should be observed in evaluating the result of such search:

(1) In the drug field, more than perhaps in any other, certain descriptive syllables are commonly used as part of trade-marks. Typical are such syllables as, for instance, "vita" for vitamin products; or "dent" or "derm," or "sulfa" or "seltzer." If the result of the search reveals that the only similarity between the name of the new product and existing registrations consists in the presence in both of such a common syllable, the applicant's mark should, as a result, qualify for registration if the remaining part of the mark is distinctly different. Thus, it has been held that "VITAMILES" did not conflict with "VITABUILD" for vitamin products, and that "ALKA-SELTZER" could not successfully oppose "PEPSO-SELTZER." Other illustrations are "ONYXSAN" and "DERMA-SAN"; "INGRAVIDULES" and "VITULES"; "POLI-GRIP" and "PERMA-GRIP"; "PEPSODENT" and "PEARLE-DENT."

(2) Even though a syllable or two may be descriptive or generic, such as "vita" or "sulfa," they will not be completely disregarded in comparing existing marks with marks sought to be registered. In other words, in order to determine likelihood of confusion, the respective marks will be considered in their *entirety*. It is highly important, therefore, that every effort be made to make the nondescriptive part of the new mark as different as possible from the nondescriptive portion of a previously registered or used mark of a competitor, so that it could not possibly be said that the marks either look or sound alike.

(3) In determining likelihood of confusion, equal consideration will be given by the Patent Office and the courts to similarity in appearance, sound, or meaning. If similarity exists in any one of these respects, the new applicant's mark is likely to be rejected.

(4) In determining likelihood of confusion between two marks, both the Patent Office and the courts will also consider general or specific differences in the goods involved. In other words, where two marks might be held confusingly similar in case the goods were identical, they may not be so held if there are marked differences in the goods in addition to differences in the marks. To put it differently, it is well established that the cumulative differences between the marks and the goods will be considered in borderline cases, where differences in goods or marks alone might be insufficient to justify a finding of confusion. An illustration of the application of this doctrine of cumulative differences may be found in a case in which the word "LIX" was registered for an insecticide over the opposition by the owner of "VICKS" for a medicated salve.

(5) In case of doubt, the Office and the court will decide against the "newcomer" in the field.

(6) The newcomer may not ordinarily rely on the fact that third parties have been granted registrations which may seem closer to the opposing party's mark than the one which the newcomer seeks to register. Such third party registrations may be referred to to show the inherent weakness of the opposing party's mark, but are not ordinarily sufficient to overcome the adverse effect of such registration on a pending new application.

These are but a few general yardsticks which an applicant for a trademark for a new drug product may use in determining whether to go ahead with the registration of a mark which has been objected to as confusingly similar to a previously registered or used mark, either by the Patent Office or by an opposing party.

III. Is Federal Trade-Mark Registration Worthwhile?

In view of all these potential difficulties and expenditures, a manufacturer may well ask himself whether registration of his new mark at the Patent Office is indispensable from a legal point of view, or at least suffi-

ciently advantageous to warrant the effort. The answer to this question, since the Trade-Mark Act of 1946, should be unqualifiedly in the affirmative. *It is, of course, still true that trade-mark rights in the United States are created by and based on use of the mark rather than on registration, and it is quite possible, therefore, that a manufacturer may decide to rely on his common law rights based on first use of the trade-mark rather than* on Federal registration. However, registration on the Principal Register of the Act of 1946, once obtained, results in a great many important benefits, such as these:

(1) Registration creates a *prima facie* presumption of validity and ownership of the mark. Under the Act of 1946, the registrant's right may become "incontestable," which means that the mark will then be completely immune against certain attacks based either on lack of priority of use on the part of the registrant, or some technical defects in the mark itself. On the other hand, it cannot be overemphasized that even incontestability will not protect a mark against becoming "generic" or, as the act calls it, "a common descriptive term." It is up to the registrant, therefore, as will be mentioned under IV, to protect his registered mark at all times against the inherent threat of becoming a generic term.

(2) Registration now serves as a "constructive notice." That is to say, no one may thereafter in good faith acquire rights in the same mark anywhere in the territory of the United States in interstate or foreign commerce, regardless of whether the registrant himself may have used the mark in that particular territory in such commerce.

(3) The registrant may deposit a copy of his registration certificate with the Customs authorities and thereby prevent importation of any merchandise bearing an identical or confusingly similar trade-mark.

(4) Where registration and protection in foreign countries is needed, registration in the United States is frequently a condition *sine qua non* for securing protection abroad.

(5) The owner of a registered trade-mark is entitled to sue in the Federal courts and ask for destruction and delivery up of all articles bearing an infringing mark, and may get triple damages in case of proven infringement.

Wherever possible, the manufacturer should seek registration on the Principal Register since registration on the so-called Supplemental Register confers none of these benefits, except that it may serve as basis for foreign registrations (although even that is no longer true in all countries; Canada, for instance, has refused to consider such registrations as a sufficient proof of actual protection in the United States), and that the jurisdiction of the Federal courts may be invoked even on the basis of such registrations.

It would seem obvious from this brief summary that most manufacturers would be ill advised today not to take advantage of the provisions of the Trade-Mark Act of 1946, and rely on common law rights only, or perhaps on some local registration statutes within one or more of the forty-eight states.

IV. Preservation of Trade-Marks

One more word should perhaps be said with regard to preservation of registered marks once registration has been secured at the Patent Office under the Act of 1946. As far as the Patent Office is concerned, there are but a few steps necessary to keep such registrations alive perpetually. Under the Act of 1946, it is necessary for the registrant to file an affidavit of continued use of the mark during the sixth year following the date of the registration. If, for special reasons, such as war or shortages, the mark is not in use during the critical period, an affidavit of "special circumstances" may be filed. Within six months of the expiration of the twenty-year registration period, an application for renewal may be filed upon payment of a fee of $25.00. A renewal application must be accompanied by an affidavit stating that the mark is still in use in commerce. These are about the only formalities which a registrant has to observe in order to keep his registration alive at the Patent Office.

Much more important for practical purposes are certain precautions which any trade-mark owner, and particularly the owner of a drug trademark, must observe outside the Patent Office in order to preserve the integrity of the mark.

(a) Since, under the statute, a period of nonuse for two consecutive years constitutes *prima facie* abandonment, any discontinuance of use for so long a period should be avoided.

(b) Any generic use by others, be it in scientific literature, such as, for instance, the major portion of this book, patent applications, or anywhere else, should be strenuously objected to, so that it could never be said that the mark has ceased to be a trade-mark because by "an act of omission or commission" on the part of its owner it has lost its significance as a trademark. Many valuable trade-marks have been lost because their owners permitted the marks to become common descriptive terms, particularly where the article was originally produced under a patent (such as, for instance, aspirin or cellophane).

(c) The owner of the mark himself should never use or refer to his own trade-mark without emphasizing its trade-mark character through use of the statutory registration notice "Reg. U. S. Pat. Off." or " ®," and without educating the public and the profession to the effect that there is another generic term for the same product available. This usually requires careful scrutiny of trade publications, newspapers and other literature.

As stated at the beginning of this article, the question whether or not the manufacturer of a new antibiotic should adopt any trade-mark in addition to the new scientific name of the product and in addition to the use of his own house-mark, is a matter of general advertising and business policy rather than a legal problem. However, if and when time and effort have been spent to develop and publicize a new trade-mark, in addition to a new generic name or in addition to the chemical name of the product, extreme care should be taken to prevent such trade-mark from becoming a generic term or from being referred to as the common or usual name under the Food, Drug and Cosmetic Act.

One final word about changes in the mark or classification after registration. Trade-marks for antibiotics are currently classified in the Patent Office in Class 18, which covers medicines and pharmaceutical preparations. Until recently, such products were covered in Class 6, which also covered chemicals, chemical compositions, and cosmetics. Such classification is, of course, only for the convenience of the Patent Office and for search purposes. If, however, a new line of goods were added, or if registration in an additional class is desired, a new application should be filed. The same is true if changes in the mark itself are made; for instance, if certain letters are added or omitted or the style of lettering is changed. Minor changes in appearance which do not constitute "a material alteration" may now be made by way of amendment without the need of filing a new application, but changes in the nature of omitting or adding letters to the mark are always considered material changes requiring reregistration.

It is not necessary to postpone filing an application for registration of a new trade-mark until such time as a new drug application may become effective. Nor does there exist a statutory provision such as prevails in cases of liquor, meat products, and insecticide labels, which would prohibit the use of a trade-mark or label in interstate commerce until after a Federal government agency has given its approval to the use of the label. Consequently, while the Patent Office will not entertain an application for registration of a liquor trade-mark unless the applicant shows approval of the label by the Alcohol Tax Unit, the Office will act upon an application for a trade-mark for an antibiotic or any other drug product, regardless of whether the manufacturer has satisfied the requirements of the Food & Drug Act with regard to certification, new drug application, or otherwise.

On the other hand, the law does not permit the filing of a trade-mark application on a "tentative" basis or on a statement of a mere intention to use the mark. Great Britain, Canada and other countries accept such declarations of intention and the combined Trade-Mark Bureau of the A.D.M.A. and A.P.M.A. makes provision for the recordation of such "tentative" trade-marks. Under the Federal Trade-Mark Act, however, an applicant must state that the mark sought to be registered is in actual use in interstate and foreign commerce. In actual practice, this requirement of use of the mark before the filing of the application has been liberally construed; for instance, use of a mark on sample shipments would be sufficient under the registration statute. In other words, once a new product has been distributed, even to a small extent, among physicians or hospitals for a trial period, this will satisfy the use requirement of the registration statute, regardless of whether the product may have found Council acceptance, or may have become effective as a new drug under Section 505(a) of the Food, Drug & Cosmetic Act.

V. Bibliography

For more detailed information with regard to the registration of Trade-Marks under the Act of 1946, the reader is referred to "Rules of Practice in Trade-Mark Cases," U. S. Government Printing Office, Washington 25, D. C. (forty cents). There is also available free of charge a pamphlet called "General Information Concerning Trade-Marks," obtainable from the U. S. Patent Office, Department of Commerce, Washington 25, D. C., upon request.

The following articles may be of interest to drug manufacturers:

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ACTIDIONE

First described by Whiffen, Bohonos and Emerson in 1946 [1]; isolated and named by Leach, Ford and Whiffen in 1947,[4] named from ACTINOMYCETE and DIKETONE (based on originally proposed diketone structure) derived from streptomycin-producing strain of *Streptomyces griseus;* registered trade-mark as "ACTI-DIONE."

PRODUCTION

Fermentation. Submerged growth in shaker flasks on medium containing g/l cerelose 10, soybean oil meal 10, "Curbay E-G" 5, $CaCO_3$ 1, NaCl 5; incubated 72 hours; average yield 232 γ/ml.[6]

Isolation. (A) Culture liquor acidified to pH 2, filtered, actidione adsorbed on activated carbon, eluted with 80% acetone, acetone removed by distillation *in vacuo.*[1]

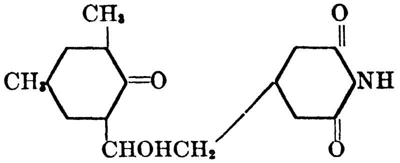
(B) Culture filtrate or acetone-free eluate extracted with chloroform, yielding orange-brown or intense green extracts depending on culture medium, decolorized by activated carbon, chloroform removed *in vacuo;* crude product orange-brown sticky oil having moldy odor; yield 48% of activity in culture liquor, 59% pure by bioassay.[2]

Purification. (A) By countercurrent distribution.[1]

(B) By carbon chromatography over activated carbon and filter-aid using 20% acetone for charging and developing column and 60–100% acetone for elution; yield 40.5% of crystalline product from crude actidione.[2]

CHEMISTRY

Constitution. Empirical formula originally suggested: $C_{27}H_{42}N_2O_7$ [1]; recent evidence supports $C_{15}H_{23}NO_4$ [2, 3, 7];



M.W. 420–275 (cryoscopic in benzene varying with concentration), 254 (camphor-Rast), 281 calculated.[1, 2]

Properties. Colorless plates m.p. 115–116.5° C (recrystallized from amyl acetate), 115–117° C (further recrystallized from water or 30% methanol), soluble in water, chloroform, ether; thermostable; $[\alpha]_D^{25} = -2.8°$ ($c = 9.6$ methanol), $-3.0°$ ($c = 10$ methanol), $-6.8°$ ($c = 2$, water).[2]

Derivatives. Semicarbazone analysis agreeing with $C_{16}H_{26}N_4O_4H_2O$, m.p. 182–183° C (from methanol), about one-twentieth as active as actidione; *acetate*, m.p. 148–149° C (from hot water), and *oxime*, m.p. 203–204° C (dec.) (from methanol) were inactive.[2]

BACTERIOLOGY

Assay. Paper-disk plate method with *Saccharomyces pastorianus;* zone size greatly influenced by inoculum concentration.[1, 6]

Spectrum in vitro. *Bacterial:* 12 species, no inhibition at 1 mg/ml.[1]

Yeasts[6]

Test Organism	mg/ml Inhibiting
Nematospora phaseoli	0.17
Pichia membranaefaciens	0.17
Saccharomyces carlsbergensis	0.17
Saccharomyces ellipsoideus var. burgundy	0.17
Saccharomyces fragilis	0.17
Saccharomyces pastorianus	0.17
Schwanniomyces occidentalis	0.17
Sporobolomyces salmonicolor	0.17
Torulaspora fermentati	0.17
Rhodotorula glutinis	0.31- 0.8
Hansenia apiculata	0.62
Hansenula anomala	2.5
Saccharomyces cerevisiae	10.0 - 0.4
Torula utilis	10.0
Asparomyces urae	25.0
Debayomyces globosum	25.0
Schizosaccharomyces pombe	25.0
Endomyces magnusii	>1,000
Kloeckera apiculata	>1,000
Mycotorula roseo-corralina	>1,000
Pityrosporum ovale	>1,000
Saccharomyces lactis	>1,000

Fungi[6]

Cryptococcus neoformans	0.08- 0.24
Phialofora verrucosa	12.5 -50
Monosporium apiospermum	25.0 -50
Hormodendrum pedrosoi	50
Blastomyces dermatitidis	1,000
Candida albicans	>1,000
Coccidioides immitis	>1,000
Geotrichum sp.	>1,000
Hormodendrum compactum	>1,000
Nocardia asteroides	>1,000
Sporotrichum schenkii	>1,000
Trichophyton rubrum	>1,000
Trichophyton mentagrophytes	>1,000
Epidermophyton floccosum	>1,000

Resistance. *Saccharomyces pastorianus* increased in resistance from inhibitory concentration of 0.06 γ/ml to 4.08 γ/ml in 3 transfers.[6]

PHARMACOLOGY

Toxicity. LD 50 (mg/kg):

mice	intravenous	150
guinea pigs	subcutaneous	60
rabbits	intravenous	17
cats	intraperitoneal	4
rats	subcutaneous	2.7
rats	intravenous	2.5

Crude product highly irritating to skin [1, 6]; caused vomiting in dogs, nausea in humans.[8]

Blood levels. In rats, 1–10 mg/kg administered intraperitoneally appeared in the blood at 5–20 units/ml; similar results with guinea pigs and dogs.[8]

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ACTINOMYCES LYSOZYME

First described by Kriss in 1940; derived from *Streptomyces violaceus;* classified as a lysozyme by the author.

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ACTINOMYCETIN

First described by Welsch in 1941 [1]; derived from *Streptomyces albus.*

This bacterial product is characterized chemically by protein-like properties, and bacteriologically by lytic action toward dead bacteria and to some

extent toward living bacterial cells.[2] (The appended bibliography is limited to the action of actinomycetin on living bacteria.)

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ACTINOMYCIN

First described by Waksman and Woodruff in 1940[1, 2, 3]; derived from *Streptomyces antibioticus;* also from other *Streptomyces* sp.[4, 5, 6, 7]; synonym ACTINOMYCIN A. Originally described as consisting of two components, ACTINOMYCIN A and B; later evidence indicated ACTINOMYCIN B is an antibiotically inactive oil contaminated by traces of ACTINOMYCIN A.[4]

PRODUCTION

Fermentation. Surface growth with *S. antibioticus* on medium containing (A) g/l starch 5, "Tryptone" 10, K_2HPO_4 2, NaCl 2, agar 15; incubated at 28° C for 7–9 days; yield 70–126 mg/l[8]; (B) The same medium but with agar 0.25%; Incubated at 25–35° C for 6–10 days; yield about 100 mg/l[9]; (C) "Starch-Tryptone" medium with 0.25% agar; yield 300 *B. subtilis* units/ml; from a *Streptomyces* sp., 3,000 *B. subtilis* units/ml[4]; (D) With *Streptomyces* sp., "Tryptone" 5, glucose 10, K_2HPO_4 2, NaCl 2, agar 2.5; incubated at 26–27° C for 6–8 days, final pH 8.0–8.2; yield 2,000 *Staph. aureus* units/ml.[7]

Submerged growth with *S. antibioticus* in shaker flasks and aerated tanks on starch-tryptone medium; yield 200 *B. subtilis* units/ml; from a *Streptomyces* sp., 3,000 *B. subtilis* units/ml.[4, 10]

Isolation. (A) Culture filtrate extracted with ether, the ether extract evaporated to dryness and the residue dissolved in alcohol.[8] (B) Culture filtrate extracted with ether, the ether extract evaporated to dryness, the residue extracted with petroleum ether and the extract discarded; the residual material dissolved in alcohol and diluted with water.[9, 10]

Purification. Crude actinomycin dissolved in benzene, chromatographed on aluminum oxide, developed with acetone-benzene, eluted with 30% acetone in benzene; the eluate evaporated to dryness and the residue recrystallized from acetone-ether or ethyl acetate.[9, 10]

CHEMISTRY

Constitution. Proposed $C_{41}H_{56}N_8O_{11}$, $C_{37}H_{50}N_7O_{10}$, or $C_{38}H_{49}N_7O_9 \cdot 1/2\ H_2O$; found C 59.01, H 6.81, N 13.35, $—OCH_3$ 0.0; $—NCH_3$ 6.05, 6.32; $—CCH_3$ 6.19, 7.72; $—COCH_3$ 2.85, 2.88; M.W. 1,000 ± (Rast in CBr_4), 768–780 (cryo. in cyclohexanol), 813 (cryo. in phenol); probably quinone and hydroxyl groups.[9, 10]

Properties. Red-vermilion platelets; m.p. 250° C (dec.) [9, 10]; soluble in chloroform, benzene, ethanol, acetone, hot ethyl acetate, carbon disulfide, 10% HCl; slightly soluble in water, ether; insoluble in petroleum ether, dilute mineral acids, dilute alkali [8, 9, 10]; $[\alpha]^{25}_{D} = -320° \pm 5°$; ultraviolet spectrum in ethanol, peak at 230–250 mμ; at 450 mμ, $E^{1\%}_{1\,cm} = 200$, shifting after 6 minutes to 241 mμ and $E^{1\%}_{1\,cm} = 216$, after 21 hours to 244 mμ and $E^{1\%}_{1\,cm} = 288$ [9]; adsorbed by activated carbon, "Seitz" filter pad.[8]

Reactions. Thermostable in aqueous, alcoholic, neutral and slightly acid solution; unstable in hot alkali and strong acid; inactivation in dilute alkali partially reversed by neutralization; very little loss by exposure to light [8, 9]; with concentrated sodium hydroxide, a transient purple color; negative with alcoholic ferric chloride; reduced by sodium hydrosulfite, stannous chloride or hydrogen over platinum oxide to pale yellow, reversed by exposure to air; no reaction with sodium bisulfite.[9]

Derivatives. Acetylated with acetic anhydride and pyridine; reductive acetylation with zinc dust.[9]

BACTERIOLOGY

Assay. Dilution and streak plate with *B. subtilis*,[4] with *Staph. aureus.*[7]

Spectrum in vitro.[8] Inhibition concentration of crude actinomycin (mg/ml): *E. coli* 0.2, *Aero. aerogenes* 0.2, *Br. abortus* 0.2, *Azot. vinelandii* 0.001, *Sar. lutea*, *B. mycoides*, *B. subtilis* 0.0001–0.00001.[8]

Inhibition concentration for fungi (mg/ml) [8]:

Rhizopus sp.	0.1–0.02	*Fusarium* sp.	0.1–0.02
Trichoderma sp.	0.1–0.02	*A. niger*	0.1–0.02
Penicillium sp.	<0.02	*A. candida*	>0.1
Humicola sp.	>0.1	Yeast	0.1–0.02
soil suspension	0.1–0.02		

Bacterial population in soil, milk and sewage reduced by actinomycin.[8]

With crystalline actinomycin, inhibition concentration (γ/ml) [9]:

B. mycoides	0.05–0.025	*E. coli*	1,000–2,000
B. subtilis	0.05–0.025	*Aero. aerogenes*	>2,000
Sar. lutea	0.01–0.005		

Inhibition of streptococcus and pneumococcus at 1:1,000,000; *Cl. welchii* and *Cl. tetani* at 1:60,000–100,000.[11]

Mode of action. Bacteriostatic and bactericidal.[8]

Factors affecting activity. Not affected by 10% rabbit serum.[11]

Spectrum in vivo. In mice, actinomycin gave no protection against experimental infections with pneumococcus and streptococcus; some protection against *T. equiperdum* [12]; possible action against fowl pox virus and laryngotracheitis virus.[13]

PHARMACOLOGY

Toxicity. In mice, MLD mg/kg, intravenous 1.0–2.0, intraperitoneal 0.5–5.0, subcutaneous 0.5–2.0, oral 20.0; in rats, rabbits, guinea pigs, hens and chick embryos toxicity similar to mice.[8, 11, 12]

Chronic toxicity by intraperitoneal administration of 0.025–0.10 mg/kg daily in rats and mice caused practically 100% deaths at 30 days with liver and kidney-function damage.[11]

Actinomycin does not hemolyze red cells.[11]

Physiology. Actinomycin does not influence blood pressure.[11]

Blood level — excretion. In mice, rats, rabbits and chickens, 2.5 γ/20 g intravenously disappeared from the blood stream in 15–60 minutes; 10–20% detected in urine after 6 hours.[11, 12]

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ACTINOMYCIN A *See* ACTINOMYCIN

ACTINOMYCIN B *See* ACTINOMYCIN

ACTINORHODINE

First described by Plotho, Brockmann and Pini in 1947; derived from *Actinomyces* sp.

PRODUCTION

Fermentation. In culture medium containing corn extract, sucrose or glycerol, glycine and potassium nitrate at pH 7.

Isolation. Extraction of mycelium.

CHEMISTRY

Constitution. Proposed $C_{23}H_{20}O_{10}$; contains 3 free OH groups, a quinone structure with 2OH adjacent to CO, one COOH group.

Properties. Fine red needles; no m.p.; soluble in pyridine, tetrahydrofuran, dioxane, alkaline solution; ultraviolet maxima at 531, 571 mμ in butanol.

Reactions. Blue color in alkaline solution, deep blue solution in sulfuric acid becoming red-violet on addition of boric acid; red solution in acetone, changing to violet-blue with red fluorescence on heating with boric-acetic anhydride.

BACTERIOLOGY

Staph. aureus inhibited at 1:100,000.

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ACTINORUBIN

First described by Kelner, Kocholaty, Junowicz-Kocholaty and Morton in 1946 [1]; derived from *Actinomyces* sp., named from its characteristic red mycelium.

PRODUCTION

Fermentation. (A) Surface growth on medium containing g/l "tryptone" 5, Na_2HPO_4 1.2, KH_2PO_4 0.8, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, sucrose 10, agar 2.5; pH 6.8–7.2; incubated at 28–30° C for 8–10 days, final pH 7.8; yield 200–400 *E. coli* units. (B) Submerged growth in shaker flasks on above medium but with sucrose replaced by molasses 10 ml; incubated 2 days, final pH 7.0; yield 200–400 *E. coli* units.[2]

Isolation. Culture liquor adjusted to pH 7, "Decalso" cation exchanger added and stirred, the supernatant liquid decanted; the sediment washed with water and acetone, dried at 28–30° C, eluted with saturated NaCl, eluate evaporated to dryness in vacuo; the residue extracted with boiling absolute methanol, the methanol extract refrigerated, a gummy precipitate separated and discarded, ether added precipitating crude actinorubin; dried *in vacuo to a brownish hygroscopic powder; yield* 82.5% of activity in culture liquor.[3]

Purification. Crude actinorubin dissolved in 85% methanol, passed through an alumina column, aqueous sodium helianthate added to the percolate, and the mixture refrigerated precipitating a crystalline addition compound; recrystallized from aqueous methanol; hydrochloride purified by chromatography.[3]

CHEMISTRY

Constitution. *Of helianthate addition compound:* by analysis C 51.38, 51.37, 51.48; H 5.80, 6.07, 5.94, 5.91; N 17.38, 17.26, 17.51, 17.29; S 8.92, 8.82, 7.33, 7.35.

Properties. *Of helianthate addition compound:* m.p. 206–214° C (corr., dec.); *of free base:* positive biuret; reduces Fehling and potassium permanganate; negative Sakaguchi and Molisch; dialyzes through cellophane; thermostable in neutral solution; stable when adsorbed on "Decalso," in NaCl eluate and methanol solution.[4]

BACTERIOLOGY

Assay. Serial dilution by streak test with *E. coli;* crude actinorubin 0.5–0.6 γ/unit, helianthate 0.5 γ/unit.[3]

Spectrum in vitro. Inhibition concentration (units/ml) [2]:

E. coli	1.05	*Diplo. pneumoniae*	>128
Aero. aerogenes	0.25	*E. typhi*	0.25–0.5
Alk. fecalis	2	*E. communior*	0.5
B. anthracis	2–4	*G. tetragena*	0.03
B. cereus	32	*K. pneumoniae*	0.125
B. mesentericus	0.5	*Micro. aurantiacus*	0.007
B. mycoides	0.25–16	*Myco. smegmatis*	1.0
B. subtilis	0.01–8	*Myco. tuberc. bov.*	8
C. diphtheriae	0.015	*Neis. catarrhalis*	0.03
C. xerose	0.004	*Pr. vulgaris*	1
Br. abortus	4	*Ps. aeruginosa*	32
Br. melitensis	8	*Sarcina lutea*	0.007
Br. suis	16	*Ser. marsescens*	4
Ch. violaceum	0.015	*Staph. aureus*	0.06
V. comma	0.5	*Strep. pyogenes*	128

S. enteriditis	0.25	*Streptococcus* alpha	>128
S. paratyphi	0.25	*Streptococcus* gamma	128
S. schottmuelleri	0.125	*Shig. dysenteriae*	1
Trich. interdigitale	8	*Shig. paradysenteriae*	1

Factors affecting activity. In 0.8% NaCl, activity is reduced to 1/128; no further effect by the addition of 10% horse blood.[2]

Spectrum in vivo. In mice intraperitoneally, 13.7 γ of actinorubin (0.2 γ per *E. coli* dilution unit) gave complete protection against experimental infection with *K. pneumoniae,* 30 γ gave 50% protection in another trial.[4]

PHARMACOLOGY

In mice, intraperitoneally, the LD 100 was 1.37 mg; the LD 50 was 0.68 mg in a second trial. Actinorubin was detected in the blood following intraperitoneal, but not following oral administration.[4]

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AEROSPORIN *See* POLYMYXIN

ALLICIN

First described by Cavallito and Bailey in 1944.[1] It has been suggested that the name "allicin" be dropped in view of possible confusion with certain well-established medicinal products.[3]

Allicin is derived from garlic cloves, but it is not normally present in the free state. Its precursor is ALLIIN, which is biologically inactive, odorless, and thermostable. When the garlic cells are crushed, alliin is rapidly broken down to yield allicin, pyruvic acid, and ammonia. The conversion takes place only in the presence of an enzyme, alliinase, also present in garlic. Allicin has the characteristic odor of garlic and is biologically active. It yields allyldisulfide, which is biologically inactive and is the chief component of "essential oil of garlic." The suggested sequence of steps is: alliin (with enzyme) yields allicin, which on loss of oxygen yields allyldisulfide.[3, 6, 7, 10, 11]

Another antibiotic, ALLISTATIN, consisting of ALLISTATIN I, II and possibly a third fraction, has been described with properties similar to those of allicin.[8]

PRODUCTION

Isolation. (A) Mixture of ground garlic cloves and 95% alcohol stirred, filtered, extract concentrated in vacuo until most of the alcohol is removed, alcoholic distillate discarded; distillation continued in vacuo, aqueous distillate collected while maintaining volume in distillation flask by addition of water from dropping funnel; distillation continued until residual solution contained less than 10 μ/ml active principle; aqueous distillate extracted with ethyl ether, and ether removed from combined extracts by distillation in vacuo; residue consisting of some water and oil shaken with water and "Shellysolve B"; aqueous solution after separation and filtration frozen and stored in "Dry Ice."[1]

(B) Whole garlic cloves ground with powdered "Dry Ice" under acetone, the solid filtered off, washed several times with acetone and dried at 70° C, giving a white odorless powder, yield 30% of the whole garlic; small quantity of water added to the powder, and active principle extracted and isolated from the mixture.[3]

(C) Mixture of crushed garlic and absolute alcohol left to stand overnight, filtered, filter cake extracted with absolute alcohol, combined extracts distilled in vacuo, alcohol-free concentrates extracted with chloroform, extract evaporated in vacuo at 30° C after drying over sodium-sulfate; yield 15 g from 4 kg garlic.[4]

Purification. Aqueous concentrate repeatedly extracted with ether, combined extracts cooled in "Dry Ice," ice crystals filtered off, ether removed in vacuo, residual oil dried at 0.5 mm or less for 30 min. at room temperature; yield about 6 g of oil from 4 kg garlic.[1]

CHEMISTRY

Constitution. Allicin is the allyl ester of allylthiosulfinic acid [2,6,7]; $C_6H_{10}OS_2$, M.W. 162; M.W. 167 (cryoscopic); synthesis by oxidation of diallyl sulfide with hydrogen peroxide in glacial acetic acid.[7]

Properties. Colorless liquid decomposing on dry distillation; adsorbed by activated carbon [9]; soluble in water (about 2.5% at 10° C), alcohol, benzene, ether, slightly soluble in "Shellysolves"; d^{20} 1.112, n_D^{20} 1.56, optically inactive, irritating to skin, has characteristic odor of garlic.

Reactions. In aqueous solutions, pH about 6.5; oil precipitated on standing; acidity slowly increased with formation of small amounts of SO_2 and activity of solution decreased; immediately inactivated by alkali [1]; alkaline hydrolysis of allicin yielded SO_2 and an insoluble oil, apparently allyldisulfide [1,2]; non-alkaline, aqueous or non-aqueous solutions or dry preparations on standing at room temperature yielded in about 2 days an inactive viscous liquid (hydrogen peroxide inhibited formation in aqueous solution) which was not water-soluble and cannot be distilled, M.W. about 485 (cryoscopic).[2]

Reacted rapidly in aqueous solution with cysteine at pH 6 to yield white crystalline precipitate soluble in acids and bases, slowly soluble in hot water; lost antibacterial activity when treated with NaCN or cysteine at pH 6; reacted with N-acetyl cysteine but not with S-methyl cysteine; rapidly decolorized potassium permanganate solution and bromine water; white precipitate with mercuric chloride; rapidly inactivated by sodium hydrosulfite; no immediate color change with Grote's reagent, but evanescent green color appears in a few minutes; hydrogen peroxide did not cause rapid inactivation; not rapidly decomposed in pyridine (aq. or anhyd.); oxidizes hydriodic acid.[2]

BACTERIOLOGY

Bacterial spectrum in vitro. Inhibition concentration in millimoles per ml (× 10,000).[1, 12]

Staph. aureus	0.6	*Sal. paratyphi*	1
Staph. albus	1.5	*Diplo. pneumoniae*	2
Sarcina lutea	0.2	*Eb. typhimurium*	1
Strep. fecalis	2	*Clos. welchii*	1
Strep. hemolyticus	1	*Clos. histolyticus*	0.6
B. subtilis	0.3–0.5	*Myco. tuberc. ranae*	0.5
B. cereus	0.3	*Asp. niger*	0.6
E. coli	1.5	*Pen. notatum*	0.9
Sh. dysenteriae	0.3	*Trychophyton gypseum*	0.06
Eb. typhi	1	*Microsporon audouini*	0.6

Bacteriostatic activity of approximately the same order are shown by the methyl, ethyl, *n*-propyl, isopropyl, *tert.*-butyl ethyl, *n*-butyl, and *n*-amyl thiolsulfinates,[12] and by related compounds formed by the enzymatic action of alliinase.[11]

PHARMACOLOGY

LD 50 in mice in aqueous solution approximately 60 mg/kg intravenous and 120 mg/kg subcutaneous.[1]

MISCELLANEOUS

Allicin inhibits SH groups of papain and the milk-clotting enzyme of *Ficus carica* at 0.2 mg/ml; can be assayed in the range of dilutions 1:20,000 to 1:300,000 against clotting time.[5]

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ALLISTATIN GROUP *See* ALLICIN

ALTERNARIC ACID

First described by Brian, Curtis, Hemming, Unwin and Wright in 1949; derived from *Alternaria solani* and possibly from *A. porri.*

PRODUCTION

Fermentation. On Czapek-Dox medium with 10–15% sucrose.

Isolation. Culture filtrate adjusted to pH 3.5, extracted with chloroform; chloroform extract evaporated to a gummy residue, dissolved in hot carbon tetrachloride or benzene; solution cooled, precipitating crystalline alternaric acid; yield 100 mg/l.

Purification. Recrystallization from benzene, followed by repeated crystallization from ethanol or water.

CHEMISTRY

Colorless crystals, m.p. 134° C; optically inactive; a dibasic acid.

BACTERIOLOGY

Assay. By "stunting" of developing germ tubes in *Botrytis allii* or *Myrothecium verrucaria.*

Spectrum in vitro. Negligible antibacterial activity. Specific antifungal activity: at 0.1–1.0 γ/ml, inhibition of spore germination of *Absidia glauca, Myrothecium verrucaria* and *Stachybotrys atra;* at 200 γ/ml, no effect on spore germination of *Botrytis allii, Fusarium coeruleum* and *Penicillium digitatum,* but "stunts" germ tubes at much lower concentration, 0.01 γ/ml being effective on *B. allii* germ tubes.

At 5–10 γ/ml, causes wilt and death in radish, cabbage, mustard and carrot seedlings, but not in tomato, pea, beet seedlings; at 1–5 γ/ml, causes retardation of growth in radish, mustard and tomato seedlings, but not in wheat and clover.

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ALVEIN

First described by Gilliver, Holmes and Abraham in 1949; derived from *Bacillus alvei.*

PRODUCTION

Fermentation. Surface growth unsuccessful unless mechanical support is provided for the pellicle, *i.e.,* cotton pads floating on the surface.

Submerged growth in aerated bottles on medium containing g/l glucose 10, corn steep liquor 10 ml, $FeSO_4$ 0.01, $NaNO_3$ 3, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, pH 7.0; incubated at 37° C for 40–48 hours; air ratio of air volume per minute per volume of culture liquor 1.0; final pH 6.6–7.0; yield equivalent to 1:80,000 of standard alvein hydrochloride. (With higher proportions of corn steep liquor and glucose, another antibiotic is formed, active against *M. phlei* but not *Staph. aureus.*)

Isolation. (A) Culture filtrate at pH 9.0 extracted with butanol. (B) Culture filtrate activity adsorbed on activated carbon at pH 7.0; activity eluted with acid aqueous butanol, the butanol separated, ether and water added, the aqueous phase separated and neutralized.

Purification. Alvein precipitated with picric acid at 4° C, the precipitate dried, converted to the hydrochloride with acid alcohol precipitated from dry ether; yield 20–30% ca. 90% pure by countercurrent analysis.

CHEMISTRY

Constitution. By analysis, C 49.0, H 8.7, N 12.9, S 1.2, Cl 8.9; probably a basic polypeptide, containing arginine, cystine, lysine, serine, threonine, alanine, valine and leucine.

Properties. Free base increasingly insoluble above pH 7.0 in water, solubility decreased by presence of sodium chloride; soluble in ethanol and butanol. Hydrochloride soluble in water and ethanol, insoluble in acetone, chloroform and ether.

Reactions. Thermostable at pH 2–7, less stable above pH 9 and below pH 2; *inactivated by trypsin.*

BACTERIOLOGY

Assay. Cylinder plate with *Staph. aureus.*

Spectrum in vitro. Inhibition dilution (× 1,000) [1]:

Staph. aureus	512	*E. coli*	32
St. pyogenes	128	*Ps. aeruginosa*	16
C. diph. gravis	128	*B. anthracis*	512
C. xerosis	8,000	*Myco. phlei*	32,000
Myco. tuberculosis	10–100		

Activity unaffected by horse serum; bactericidal to *Myco. tuberculosis;* no increase of resistance with *Staph. aureus.*

PHARMACOLOGY

Hemolytic to red blood cells.

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ANEMONIN

Antibacterial action first described by Boas 1934,[5] [but cf. history of anemonin [6] cf. PROTOANEMONIN]; derived from *Anemone pulsatilla, A. pratensis, Ranunculus acer, R. japonicum, R. sceleratus, R. arvensis, R. alpester. R. bulbosus, R. montanus* [1, 5, 6].

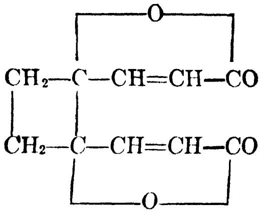
PRODUCTION

Isolation. Macerated fresh plants steam-distilled, distillate saturated with NaCl, extracted with ether, benzene or chloroform, extract evaporated in vacuo to yield a pale-yellow, irritating oil (protoanemonin) which polymerizes at room temperature within a few hours to form crystalline anemonin; yield 0.012% of fresh plant in *R. japonicum* [1, 3, 4]; also prepared by synthesis, by steam distillation of protoanemonin.[1, 2, 9]

Purification. Recrystallized from chloroform, dissolved in boiling alcohol, filtered, alcohol solution concentrated yielding crystalline material, recrystallized from chloroform; yield 0.05% of fresh, 0.23% of dry plant.[3]

CHEMISTRY

Constitution. $C_{10}H_8O_4$[12]



Structure confirmed by mixed melting point with synthetic anemonin.[1]

Properties. Crystals or plates as obtained by polymerization from protoanemonin; fine prisms, plates, rhomboids or needles when recrystallized from alcohol; colorless, odorless, tasteless; neutral reaction; crude crystals m.p. 152° C, recrystallized from alcohol m.p. 158° C; non-volatile on steam distillation [1]; volatile on steam distillation [6]; slightly soluble in cold water and alcohol; more soluble in hot water and alcohol; soluble in chloroform, fatty oils, aqueous alkali; insoluble in ether.

Reactions. Polymerizes on exposure to air; partially depolymerized by heat into protoanemonin [1]; reduces gold and platinum chloride, silver nitrate, Fehling; deep yellow color with aqueous alkali.[6]

BACTERIOLOGY

Inhibition dilution against *Mucor stolonifer* 21,000–70,000, *Aspergillus niger* 25,000–50,000, *Saccharomyces cerevisiae* 50,000–100,000, *Mycoderma* and *Oidium lactis* 30,000; no activity against *E. coli*, *C. albicans*, *Staph. aureus* [3, 4, 5, 7, 8]; inactivated by cysteine.[8]

PHARMACOLOGY

In aqueous solution 2 mg/ml at pH 3.5–4.0 causes irritation of throat.[4]

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ANTIMYCIN A

First described by Leben and Keitt in 1947 [1,2]; derived from *Streptomyces* sp., named from its fungicidal properties. Although antimycin B, etc., has not been as yet described, there is evidence indicating the presence of other antibiotic substances in crude preparations.[4]

PRODUCTION

Fermentation. Surface growth on medium containing g/l extract from 40 g potatoes, glucose 5, agar 17; incubated at 24–28° C.[3]

Submerged growth in shaker flasks on medium containing g/l corn steep liquor (50% solids) 50, glucose 10, calcium carbonate 1.5; pH 7.0; incubated at 26° C for 3–4 days; final pH 8.0–8.5; yield 1–3 units/ml.[3]

Submerged growth in iron tank on medium containing g/l soybean oil meal 40, glucose 20, calcium carbonate 1.5; pH 7.0; incubated at 24–28° C for 80–96 hours with continuous stirring, aeration rate at 50–100 liters per minute for 70 liters of culture medium under air pressure of 10–15 psi; "D.C. Antifoam A" in soybean oil; final pH 7.6–7.8; yield 200–400 units/ml,[4] 3.5 units/ml.[3]

Isolation. (A) Culture filtrate acidified to pH 2.5 with HCl, the precipitate extracted with absolute ethanol; water added to the alcohol extract, precipitating the active material, the precipitate extracted with water at pH 9.3.[3] (B) Culture liquor filtered at pH 9.0, the filtrate adjusted to pH 2.5 with HCl and filtered with filter aid; the filter cake washed with water at pH 2.5 and then extracted with 95 *per cent* alcohol; the alcohol extract concentrated in vacuo and the residue extracted with ether; the ether extract evaporated to a dark, oily paste.[4]

Purification. Crude material dissolved in absolute ether, filtered and the ether distilled; the residue washed with petroleum ether to remove a nearly inactive oily liquid; the residue exhaustively extracted in a Soxhlet with petroleum ether; the solids separating from the petroleum ether taken up in boiling ether, petroleum ether added and allowed to crystallize at room temperature and at 4° C; the crystals washed with petroleum ether and dried; recrystallized from methanol, aqueous methanol, isopropyl ether, isopropyl-ether-petroleum ether; yield 20% of original activity.[4]

Constitution. Proposed $C_{28}H_{40}O_9N_2$, C 61.30, H 7.35, N 5.11, M.W 548.6; found C 61.11, 61.09, H 7.26, 7.47, N 5.24, 5.24; alkoxyl nil; M.W. 506, 493 (Rast in camphor), 556, 560 (neutralization equivalent as a monobasic acid).[4]

Properties. Colorless crystals; m.p. 139–140° C; soluble in ether, lower alcohols, ethylene glycol, propylene glycol, methyl acetate, pyridine, acetone, chloroform; slightly soluble in petroleum ether, benzene, toluene, carbon tetrachloride; insoluble in water, in dilute aqueous HCl, sodium carbonate or sodium bicarbonate; adsorbed by activated carbon but cannot be eluted; nondialyzable through cellophane; $[\alpha]_D^{25} = +64.8°$ ($c = 10$ in chloroform); ultraviolet maxima at 230 and 320 mμ.[3, 4]

Reactions. Weakly acid, no basic properties; positive Millon, ferric chloride, Gibbs phenol; negative Molisch, ninhydrin, Ehrlich, fuchsin aldehyde, 2,4-dinitrophenylhydrazine, concentrated sulfuric acid; stable in alcohol; loss of activity on heating; in aqueous sodium hydroxide, disintegration of crystals to form a milky suspension and irreversible loss of activity.[4]

BACTERIOLOGY

Assay. Agar streak, paper disc with *Glomerella cingulata;* crystalline antimycin A 900–1,300 units/mg.[3, 4]

Spectrum in vitro. Of many bacterial species tested, only *Er. amylovora* and *B. cereus* var. *mycoides* were inhibited.

Inhibition concentration (γ/ml)[3,4]:

Ascochyta sp.	25.0	*Glomerella cingulata*	0.8–62.5
Cholara quercina	0.8	*Nigrospora sphaerica*	0.2
Colletotrichum circinans	1.6	*Phoma lingam*	0.4
Colletotrichum lindemuthianum	12.5	*Stemphylium sarcinaeforme*	1.6
Colletotrichum phomoides	0.8	*Sclerotinia fructicola*	0.4– 1.6
Colletotrichum pisi	1.6	*Venturia inaequalis*	0.4–08
Pythium sp.	250		
Fusarium oxysporum f. lycopersici	>250		
Fusarium oxysporum f. nicotianae	>250		
Fusarium oxysporum f. pisi	>250		

Other fungi inhibited:

Alternaria solani
Cholara quercina
Cochliobolus miyabeanus
Colletotrichum graminicolum
Colletotrichum lagenarium
Mycospaerella citrullina
Phoma terrestris
Phycomyces blakesleeanus
Pythium graminicolum
Gibberella zeae
Helminthosporium sativum
Macrosporium sp. (muskmelon)
Macrosporium sp. (pea)
Neurospora crassa
Sclerotinia laxa
Sclerotinia minor
Sclerotinia sclerotiorum
Fusarium oxysporum f. conglutinans

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ANTI-SMEGMATIS FACTOR

First described by Kelner and Morton in 1946; derived from *Actinomyces* sp.

PRODUCTION

Fermentation. Surface and submerged growth on medium containing g/l "tryptone" 5, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, Na_2HPO_4 1.2, KH_2PO_4 0.8, $FeSO_4 \cdot 7H_2O$ 0.01, glycerine 30 ml for submerged culture, glycerine 40 ml and agar 2.5 for surface culture, pH 6.8–7.2; incubated at 28° C for 7–9 days surface and 4–9 days submerged culture, with pH 6.8–7.0 and 6.0 respectively; yields 2,000–4,000 *Myco. smegmatis* dilution units.

Isolation. Culture filtrate frozen and thawed slowly, bottom layer of melted liquid separated and maintained in the frozen state.

BACTERIOLOGY

Assay. Agar streak plate with *Myco. smegmatis.*

Spectrum in vitro. Culture filtrate at 2,000 units/ml active against *B. subtilis* 1:10, *Myco. phlei* 1:400, *Myco. tuberculosis* (non-path.) 1:40. No activity against a number of bacterial and mold species including *Myco. tuberculosis bovis.*

Factors affecting activity. Stable at 100° C for 30 minutes; activity increases at increased alkalinity.

PHARMACOLOGY

In mice, 2 ml of culture filtrate containing 4,000 *Myco. smegmatis* units administered intraperitoneally was tolerated.

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ASIATICOSIDE

Derived from the Madagascar varieties of *Centella asiatica* (*Hydrocotyle asiatica*), used in India and Madagascar for the treatment of leprosy[1]; mentioned in the French Pharmacopoeia of 1884; isolated and named by Bontemps in 1941.[2] Ceylon varieties of *C. asiatica* yielded no asiaticoside, but "centoic acid"[3] and "centelloside."[4]

Antibiotic activity of OXYASIATICOSIDE, a water-soluble derivative of asiaticoside, first described by Boiteau, Dureuil and Rakoto-Ratsimamanga in 1949.[5]

CHEMISTRY

Constitution. A glycoside; proposed $C_{21}H_{34}O_8$, $C_{54}H_{90}O_{28}$ or $C_{54}H_{88}O_{23}$; found C 58.18, 58.38, H 8.32, 8.25.[2]

Properties. Colorless prisms recrystallized from dilute alcohol; m.p. 230–233° C (dec.); $[\alpha]_D = -14°$ (alcohol)[2]; insoluble in water, slightly soluble in alcohol, very soluble in pyridine.[1]

Reactions. On hydrolysis, yielded one molecule of glucose and an amorphous glucone.[2]

BACTERIOLOGY

In vitro. Oxyasiaticoside inhibited *Myco. tuberculosis* at 0.015 mg/ml.[5]

In vivo. In rats, guinea pigs and rabbits with experimental *M. tuberculosis* infection, 0.5 ml of a 4% solution of oxyasiaticoside administered subcutaneously twice a day for 68 days resulted in one-third to one-half as many tubercular lesions as in the untreated controls.[5]

PHARMACOLOGY

Toxicity. The toxic level of oxyasiaticoside was considerably less than its tuberculosis therapy level.[5]

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ASPERGILLIC ACID

First observed by White in 1940[1]; isolated and named by White and Hill in 1943[2]; derived from *Aspergillus flavus*.

Aspergillic acid, as well as other antibiotically active substances formed by *A. flavus* and related forms, has been produced and studied by many observers. In one instance, the activity isolated was named "aspergillin," and was later recognized as aspergillic acid[3]; in another,[4] no name was assigned and identification as aspergillic acid was carried out by other observers.[5] Yet another active material appears to be similar to but not identical with aspergillic acid.[6] Other observers have reported that the major proportion of the activity in the culture liquor is aspergillic acid,[3, 7] but that varying amounts of hydroxyaspergillic acid [5] and penicillin-like antibiotics are also formed.[8, 18] On tryptone medium, large amounts of aspergillic acid are formed with little of the penicillin-like antibiotics in surface culture [10]; more of the penicillin-like antibiotics are formed in submerged culture on Czapek-Dox medium with adjuvants.[18, 19] The presence of sugars in the culture medium increases the proportion of hydroxyaspergillic acid, and since the separation of hydroxyaspergillic acid from aspergillic acid is difficult, it is suggested that sugar-free media be used for the production of pure aspergillic acid.[9]

PRODUCTION

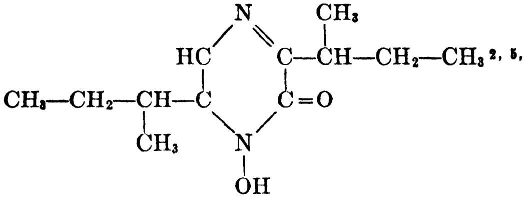
Fermentation. Surface culture on medium containing: (A) "Tryptone" 2%, NaCl 0.5% (glucose 0.5% optional), incubated 5–6 days, final pH 8.0–9.0, yield 5–70 mg/l.[2] (B) On above "Tryptone"-NaCl medium with 2–4% dark brown sugar, incubated 5–9 days, final pH 7.6–8.1, yield up to 400 mg/l,[10] but includes a large proportion of hydroxyaspergillic acid.[5] (C) On above "Tryptone"-NaCl medium with 2% dextrose incubated 8–10 days.[11] (D) On yeast extract 2%, glycerol 1%, incubated 8 days, pH rise from 6.3–6.6 to 7.9; yield 0.8–1.0 g/l, including varying amounts of hydroxyaspergillic acid and penicillin-like substances.[8] (E) On "trypticase" soy medium incubated 7 days at 25° C.[12] (F) On peptone 2%, lactose 2%, yield 500 mg/l including hydroxyaspergillic acid.[7]

Isolation. (A) Acidified culture filtrate extracted with chloroform; chloroform extract concentrated and shaken with aqueous sodium carbonate; the aqueous solution is acidified, precipitating crude aspergillic acid.[9] (B) Acidified (pH 4) culture filtrate extracted with benzyl acetate, the extract shaken with aqueous sodium bicarbonate, toxic impurities removed by treatment with activated carbon.[11] (C) Acidified (pH 4) culture filtrate treated with 2% activated carbon; carbon cake air-dried, extracted with ether in Soxhlet, ether extract evaporated to a red gum, residue extracted with aqueous sodium bicarbonate, filtered; filtrate acidified to pH 4.5, precipitating crude aspergillic acid.[2] (D) Culture filtrate concentrated to 1/3 volume, concentrate adjusted to pH 4.2 with HCl and rapidly steam-distilled to yield a pale-yellow, waxy, amorphous distillate; distillate dissolved in chloroform, evaporated to a viscous yellow residue which slowly hardens to a crystalline mass.[12]

Purification. (A) Crude aspergillic acid dissolved in boiling hexane, filtered, concentrated, precipitating near-purity aspergillic acid; redissolved in acetone or methanol, treated with activated carbon and recrystallized.[9] (B) Dissolved in alkaline solution, precipitated by acidification, crystallized from dilute alcohol.[2]

CHEMISTRY

Constitution. $C_{12}H_{20}N_2O_2$,



[2, 5, 9, 13]

Properties. Pale-yellow elongated plates or rods, with odor suggesting black walnuts; m.p. 93° C; soluble in water (1 mg/ml), dilute alkali, alcohol, ether, acetone, glacial acetic acid, benzene, chloroform, carbon tetrachloride, pyridine, excess acid; insoluble in cold water, dilute acid, petroleum ether; stable in hot alkali and acid.[2, 5, 9, 13, 14]

Acidic properties, $pK'_a = 5.3$; weak basic properties; $[\alpha]_D^{24} = +13.4°$ in ethanol ($c = 0.85$), $+18.5°$ in $1N$ NaOH ($C = 1.05$); ultraviolet maxima at 230–250 and at 300–350 mμ, varying with solvent and pH.[2, 5, 9, 13] .

Reactions. Forms soluble barium and calcium salts; copper salt, green rectangular leaflets m.p. 198–199° C; silver salt, colorless plates, darken at 170° C, decompose at 190° C; phenylhydrazine salt m.p. 99.5° C; 3,5-dinitrobenzoic acid salts m.p. 166–168° C and 123° C; hydrochloride m.p. 178° C.[9, 13]

Negative Millon, xanthoproteic, Molisch, biuret, ninhydrin, dinitrophenyl hydrazine, Fehling; no reaction with nitrous acid, potassium permanganate; no reaction with bromine in carbon tetrachloride or glacial acetic acid but vigorous reaction with aqueous bromine; intense red color with ferric chloride in alcohol; precipitated with phosphotungstic acid, pale green precipitate with copper sulfate, pale orange precipitate with cobalt chloride; crystalline precipitate with silver nitrate and mercuric acetate but not with mercuric chloride.[2, 5, 9, 11, 12, 13]

Derivatives. Desoxyaspergillic acid, antibiotically inactive but at 3 mg/ml inhibited germination of *A. flavus* spores; bromoaspergillic acid, with antibiotic activity similar to aspergillic acid; other derivatives.[9, 14, 15]

BACTERIOLOGY

Assay. Serial dilution with *Pasteurella multocida*,[12] *Str. pyogenes*,[10] *Staph. aureus*[8]; cylinder plate method with *Myco. tuberculosis*[11]; antiluminescent activity against *Photobacterium fischeri*[16]; by ultraviolet absorption spectrum.[8]

The assay for aspergillic acid is complicated by the observation (noted above) that *Aspergillus flavus* will also produce varying amounts of hydroxyaspergillic acid and penicillin-like antibiotics. Hydroxyaspergillic acid has the same ultraviolet characteristics as aspergillic acid but is only one-tenth to one-third as active. On the other hand, the penicillin-type antibiotics will influence the bioassay, but will not appear in the ultraviolet spectrum at 300–350 mμ. Consequently, for precise assay of aspergillic acid, the ultraviolet assay must be checked against the bioassay.[8]

Spectrum in vitro:

Inhibition Concentration (γ/ml) [10]

Strep. pyogenes	2	*Clos. novyi*	20
Diplo. pneumoniae	4	*Clos. perfringens*	40
K. pneumoniae	8	*Clos. septicum*	40
Staph. aureus	10	*Clos. sporogenes*	60
S. enteriditis	30	*Clos. bifermentans*	60
E. coli	30	*Clos. histolyticum*	60
Aero. aerogenes	30		

	Inhibition dilution Heavy inoculum	Lethal dilution Light inoculum[2, 11]
Staph. aureus	10,000	100,000
Strep. B hemolyt.	20,000	
Strep. fecalis	5,000	
Strep. fecalis γ		25,000
E. coli	5,000	25,000
Aero. aerogenes	10,000	50,000
Streptococcus A		25,000
Pneumococcus I		25,000
Myco. tuberculosis hominis	10,000	

Factors affecting activity. Activity is reduced by defibrinated rabbit blood, by iron[11]; activity increased by cobalt, nickel, zinc, arsenic and bismuth, the last being most effective[17]; anti-tuberculosis activity increased by cobaltous ion, particularly in iron-free media.[20]

Spectrum in vivo. No protection by intraperitoneal administration in mice against hemolytic streptococcus, pneumococcus, *Ps. aeruginosa*, *Staph. aureus;* possible protection against gonorrhea and gas gangrene.[2]

PHARMACOLOGY

MLD in mice: orally 5 mg/20 g, intraperitoneally 3 mg/20 g,[2] intraperitoneally 40 mg/kg.[3]

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ASPERGILLIN

The name "aspergillin" was originally used to describe the black pigment found in the spores of *Aspergillus niger*.[1] It has been applied to antibiotics which were later found to be gliotoxin,[2, 3] aspergillic acid and flavacin,[4, 5, 6] probably fumigacin or gliotoxin,[7] and an as yet unidentified antibiotic, possibly clavacin or aspergillic acid.[8]

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AUREOMYCIN

First described by Duggar in 1948[1], derived from *Streptomyces aureofaciens*.

PRODUCTION

Fermentation. Submerged growth in shaker flasks on medium containing corn steep liquor 1–2%, sucrose 1–3%, $(NH_4)_2HPO_4$ 0.5%, KH_2PO_4 1.5%, $MgSO_4 \cdot 7H_2O$ 0.2%, $MnCl_3 \cdot 4H_2O$ 0.00033%, $CuSO_4 \cdot 5H_2O$ 0.00033%, $ZnSO_4 \cdot 7H_2O$ 0.005%, pH 6.2; inoculated with 1% spore suspension; incubated 24–36 hours at 28° C; yield 1,000–1,500 *Staph. aureus* units/ml.

In 100-gallon glass-lined tank on medium containing corn steep liquor 1%, sucrose 1%, $(NH_4)_2HPO_4$ 0.2%, KH_2PO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.025%, $CaCO_3$ 0.1%, pH 6.0–6.1; inoculated with 0.1–2% vegetative culture; air volume per minute/culture medium volume ratio 0.5–1.5; antifoam 1% octadecanol in lard oil; incubated 24–48 hours at 26–28° C, pH falling to 4.5–4.8 during fermentation.

In deep tank fermentation also on medium containing as carbon sources starch, monosaccharoses 0.5–5%; as nitrogen sources, amino acids, casein, fish meal, soybean meal, liver cake, urea, nitrates, NH_4 compounds, corn steep liquor 0.1–5%; as inorganic salts, phosphates, K, Ca, Mg, S, Fe and trace elements.[2]

Isolation. Salted into esters, *e.g.*, methyl, ethyl or amyl acetate, into alcohols, *e.g.*, isopropyl, butyl, or into acetone by addition of ammonium sulfate.[3]

Purification. Culture filtrate activity adsorbed on "Florisil" column or on activated carbon, "Super-Filtrol," aluminum oxide, magnesium oxide; the column washed with water, and then with acetone; developed chromatographically with acid alcohol under pH 5.0; the second band, yellow under ultraviolet light, eluted, the eluate concentrated in vacuo to dryness; the residue extracted with *n*-butanol, the extract washed with water and concentrated in vacuo; absolute ether added to the concentrate, precipitating aureomycin; the precipitate washed with ether, dried, dissolved in water with HCl to pH 2–3, and freeze-dried; potency 2,900 units/mg (based on penicillin G unit).[3]

CHEMISTRY

Constitution. Free base, by analysis C 54.56, H 5.34, N 5.77, Cl 7.16 (non-ionic), O 21.17 (by diff.); M.W. 508.[25]

Monohydrochloride, by analysis C 51.49, H 5.44, N 5.53, Cl 13.51, O 24.03 (by diff.) [2]; C 51.84, H 5.24, N 5.46, total Cl 13.27, ionic Cl 6.69, O 24.19 (by diff.).[25]

Properties. *Aureomycin-free base.* Very small crystals, acicular to bladed in habit, refractive index parallel to elongation 1.674–1.694; [2] m.p. 168–169° C (uncorr.) [25]; soluble in water 0.55 mg/ml at 25° C, very soluble in water above pH 8.5; soluble in dilute acids and bases, in "Cellosolve," "Carbitol," dioxan, pyridine; less soluble in methanol, ethanol, butanol, acetone, ethyl acetate, benzene; insoluble in ether, petroleum ether [2, 4, 25]; $[\alpha]^{D}_{23} = -274.9°$,[2] -275.0 (methanol).[25]

Aureomycin hydrochloride. Clear, vitreous, lemon-yellow rhomboid crystals; refractive index 1.700 ± 0.005, acute angle 80° ± 5; decomposes above 210° C; soluble in water 14 mg/ml (approx.) at 25° C; soluble in methanol; slightly soluble in ethanol; soluble in acetone 0.13 mg/ml at 25° C; pH 2.8–2.9 in aqueous solution; $[\alpha]^{23}_{D} = -274.9°$ (methanol),[2] $-295.9°$ (water),[2] -240.0 (water) [25]; ultraviolet maxima at 255, 285 and 345 $m\mu$ in $0.1N$ sodium hydroxide, at 226, 264 and 365 $m\mu$ in $0.1M$ phosphoric acid; UV inflections vary at different pH, not sharply defined; characteristic infrared absorption spectrum.[2, 4, 25]

Reactions. With alcoholic ferric chloride, greenish-brown color by reflected light, reddish color by transmitted light[25]; forms salts with acids and alkalies; precipitates with picric acid, Reinecke's acid, ammonium molybdate; unstable on heating in aqueous strong acids and alkalies; stable at pH 2.5, unstable at pH above 7.0, particularly in culture media.[2, 4, 5, 6]

BACTERIOLOGY

Assay. The assay of aureomycin is complicated by its instability at 37° C and in the presence of broth, serum or blood.[5, 8, 9, 10, 11] Cylinder-plate method with *B. subtilis*,[5] turbidimetric with *Staph. aureus* [9, 26]; serial dilution for aureomycin in blood serum with *B. subtilis* [5]; with beta hemolytic streptococcus [7]; with *B. cereus* var. *mycoides*.[8, 9, 17] Plate assays higher and less consistent than turbidimetric.[26]

Spectrum in vitro. Inhibition concentrations are greater at 96-hour than at 24-hour incubation, due probably to decomposition of the aureomycin.[5, 6]

Complete inhibition concentration (γ/ml) [5,10]:

S. cholerae-suis	6.3	*Staph. aureus*	1.0–50
S. typhosa	3.1–50	*Staph. citreus*	12
S. typhimurium	50	*Sar. lutea*	3–50
S. pullorum	12	*Ser. marcescens*	100–200
S. derby	50	*Alkaligenes*, sp.	6
S. manhattan	50	*B. subtilis*	0.78–12.5
Salmonella sp.	3.1–25	*B. megatherium*	3.12
S. oregon	50	*Bacillus* sp.	0.78–6.25
E. coli	3.1–100	*Aero. aerogenes*	12.5–50
E. intermedia	50	*D. pneumoniae*	0.1–1.0
St. mitis	0.8–6.3	*H. hemolyticus*	0.8
St. pyogenes	0.5–1.0	*H. influenzae*	1–2
St. hemolyticus	6.25	*N. catarrhalis*	2.0
St. fecalis	6.3–50	*N. gonorrheas*	0.25–1.0
Staph. albus	1.0–2	*N. meningitidis*	0.5

Pleuropneumonia-like	0.25
Pr. morganii	4.0
Pr. vulgaris	50–250
Ps. aeruginosa	100–250
Kl. pneumoniae	6–100

No viruscidal or rickettsiacidal activity in vitro [14] (but see in vivo action below).

Factors affecting activity. Antibiotic activity reduced 55–96% by decomposition in various culture media [5]; antagonized by blood and serum [6, 26]; decreased by increase of pH from 6.1 to 8.0, by anaerobiosis, by large inocula [10, 11]; no evidence for "aureomycinase." [10, 11]

Mode of action. Bacteriostatic.[6]

Resistance. Increased resistance after 14 transfers on media with increasing concentrations of aureomycin, ratio of initial to final inhibition concentration: *Pr. vulgaris* 533, *St. hemolyticus* 66.6, *B. subtilis* 62, *K. pneumoniae* 32, *E. coli* 16.6, *S. typhosa* 4.1, *Sar. lutea* 2, *Staph. aureus* 1.0 [5]; after 42 transfers, *K. pneumoniae* 4; after 60 transfers, *Aero. aerogenes* 3.5; no increased resistance by *Staph. aureus, E. coli, Ps. aeruginosa*.[10]

Spectrum in vivo. *Experimental bacterial infections in animals:* In mice, with infective and therapeutic dose injected intraperitoneally and simultaneously:

Organism	Dose (mg)	% Protection	% Control survival [5]
S. typhosa	0.5	100	1
S. typhimurium	0.5	10	5
E. coli	0.5	100	30
K. pneumoniae	0.05	0	0
Pr. vulgaris	0.05	85	0
St. hemolyticus	0.05	65	0

Organism	mg/kg/day	% Protection	Dose [13]
S. typhosa	8	100	1/2 hr. before infection
	40	100	1/2 hr. after infection
Er. rhusiopathiae	30	100	1/2 hr. before infection
Past. multocida	10	100	1/2 hr. before infection
(intramuscular)	40	80	1/2 hr. after infection
(oral)	20	50	24 hr. after infection
S. gallinarum	120	70	for 6 days

Organism	mg/kg Subcutaneously	Hrs. after infection	% Protection	% Control survival [13]
K. pneumoniae	a 50	0	40	
	b 30	20	0	0
St. hemolyticus	a 50	0	30	
	b 15	20	50	0
Pneumococcus I	a 50	0	60	
	b 15	20	90	0

a = 1,000 LD b = 10,000 LD

Oral administration at 50 mg/kg gave no protection against Pneumococcus I and *K. pneumoniae* A, 60–90% protection against *St. hemolyticus* beta. Single doses were less effective than the same amount spread over three days[13]; in combination with streptomycin or sulfadiazine protected against *Brucella* infections[34]; no protection against tuberculosis in guinea pigs[27]; synergism with penicillin against *S. typhosa* varied from 5–40%.[5]

Experimental virus and rickettsial infections in animals. In 7-day chick embryos, 1.0 mg of aureomycin gave complete protection against the virus of psittacosis. In similar experiments, protection was observed against viruses of the psittacosis-lymphogranuloma group, rickettsiae of murine typhus, epidemic typhus, scrub typhus, Rocky Mountain spotted fever, boutonneuse fever, South African tick-bite fever, rickettsialpox, North Queensland tick typhus, Q fever.[14]

In mice, 1.0 mg/day subcutaneously, administered 24 hours after infection, gave protection against lymphogranuloma virus, murine typhus, rickettsialpox, scrub typhus.[14]

In guinea-pigs, 2–3 mg/day subcutaneously, injected 24–48 hours after infection, protected against Rocky Mountain spotted fever, epidemic typhus and Q fever.[14, 15]

Clinical trials

Oral administration of aureomycin:

Organism	Total g	Dose days	No. cases	No. favorable[16]
N. gonorrheae	1–3	1–2	66	49
N. introcellularis	4	3	1	1
D. pneumoniae	5–20	5–10	4	4
S. newport	5–7	3–8	2	1

Ocular infections:

Organism	No. cases	No. favorable[19]
Staph. aureus	105	103
D. pneumoniae	5	5
H. influenzae	4	4
Moraxella lacunata	5	4
E. coli	1	1
virus (herpes)	6	5

Other eye diseases (viruses, tubercular, of non-bacterial origin and of doubtful etiology) gave variable responses to aureomycin.[19]

Oral administration of aureomycin was effective in the following: brucellosis, epidemic typhus, urinary infections with coli-aerogenes group,[18, 33] *Staph. aureus* and *St. fecalis* infections,[18] Rocky Mountain spotted fever,[17, 18] Q fever [21]; lymphogranuloma venereum and granuloma inguinale (by intramuscular administration)[20]; amebiasis[23]; brucellosis.[28, 29, 30, 32]

Aureomycin was ineffective against typhoid,[16, 17, 18] *S. suipestifer*, *E. coli*, *Proteus*,[16, 18] *Pseudomonas*, measles.[16]

No evidence of bacterial resistance developing during therapy.[16]

PHARMACOLOGY

Toxicity [4, 5, 13]

Mice:	intravenous	LD 50	50–134 mg/kg
	intraperitoneal		200 mg/kg tolerated
	oral		1,500 mg/kg tolerated
	subcutaneous	LD 50	3–48 mg/kg
Rats:	intravenous	LD 50	118 mg/kg
	intraperitoneal		200 mg/kg tolerated
	oral		3 g/kg tolerated
Guinea pigs:	intravenous		50 mg/kg tolerated
	subcutaneous		300 mg/kg tolerated
Rabbits:	intravenous		50 mg/kg tolerated
Cats:	intravenous		50 mg/kg tolerated
	(In one animal, 40 mg/kg caused severe fall in blood pressure of cardiac cessation; a repetition of the dose was lethal.)[5]		
Dogs:	intravenous		50 mg/kg tolerated
			150 mg/kg lethal

No subacute or chronic toxicity in dogs, guinea pigs, mice, rats, chicks upon repeated intravenous and intramuscular doses and prolonged oral administration [4, 13]; no observable tissue or organ pathology.[13] No reaction to 0.25–1.0 *per cent* solution of aureomycin borate injected into conjunctival sac of rabbit eye [13]; mild irritation of eye by 0.5 *per cent* solution.[4] In animals, following subcutaneous, intracutaneous, or intraperitoneal administration, irritation, edema, necrosis at site of injection, in some cases loss of weight [4]; in dogs, intravenously, hemoglobinuria caused by HCl of aureomycin hydrochloride [4]; local erosion of gastric mucosa in mice when administered orally by intubation [13]; drug toxicity, peritoneal adhesions in guinea pigs.[27]

In humans, severe pain, inflammation, loss of appetite, nausea, occasional vomiting,[17, 18, 21] secondary anemia [19, 20]; in ointment form, irritating to eye, aureomycin borate nonirritating but loses activity rapidly.[19]

Blood levels. In animals, 20–40 mg/kg orally appeared in blood after one hour at 1.25 γ/ml; 20 mg/kg intravenously at three 2-hour intervals showed 40 γ/ml after 2 hours.[4, 13]

In humans, 0.5 g orally or 40 mg intravenously appeared in blood after one hour at up to 2.4 γ/ml; peak blood levels obtained 3 hours after intramuscular injection of 100 mg, at about 6 hours after 700 mg orally.[17]

Spinal fluid levels. A concentration of 0.8 γ/ml was detected in the spinal fluid after 20 mg/kg intravenously.[4]

Absorption, excretion. From 2–13% of orally administered aureomycin appears in the urine, 3–4 hours after administration; levels as high as 256 γ/ml have been detected.[4, 11, 18]

Physiology. Mild diuresis in rats, slight albuminuria in dogs and rabbits; fall in blood pressure due to HCl of aureomycin hydrochloride; no other physiological effect [4]; diminution of clotting time of blood.[22]

MISCELLANEOUS

Aureomycin is suggested for use in selective media for the growth of *Endamoeba histolytica*.[24]

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AUXIN

Antibacterial action of auxins first described by Dubos in 1946.[1]

BACTERIOLOGY

Spectrum in vitro. Minimal concentration per cent for 50% growth inhibition [1]:

	M.t.*	St.h.	S.s.	Sh.p.
Indoleacetic acid	0.01	0.01	0.001	0.003
Indolepropionic acid	0.0005	0.015	—	0.008
Indolebutyric acid	0.01	—	—	0.01
Naphthaleneacetic acid	0.005	0.01	0.01	0.01
Naphthoxyacetic acid	0.003	0.01	—	0.01
2,4-dichlorophenoxyacetic acid	0.003	0.015	—	0.01
Triiodobenzoic acid	0.002	0.003	0.001	0.01

* M.t. = *Myco. tuberculosis*

St.h. = *St. hemolyticus*

S.s. = *St. salivarius*

Sh.p. = *Sh. paradysenteriae*

E. coli and *Eb. typhi* inhibited by indoleacrylic acid at M/4,000–8,000[2]; anti-tuberculosis activity by 2,3,5-triiodobenzoic acid[3]; bacteriostatic at 50 mg per cent concentration.[4]

Factors affecting activity. Activity not markedly affected by inoculum concentration, nor by serum albumin; antagonized by peptone and tryptophane; increased activity in acid solution, but anti-tuberculosis activity independent of pH.[1]

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AVENACEIN *See* LATERITIIN GROUP

AYFIVIN

First described by Arriagada *et al.* in 1949[1]; derived from *Bacillus licheniformis.*

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l glucose 10, extract from 200 g of potatoes, pH 7.0; incubated at 37° C for 5–6 days, final pH 7.2; yield 4–8 arbitrary units/ml.[1] (B) glucose $0.2M$, lactate $0.05M$, ammonia $0.08M$, phosphate $6M$, citrate $1.7\mu M$, Mg^{++} $4\mu M$, $SO_4^{=}$ $4\mu M$, Fe^{+++} $70\mu M$, Mn^{++} $13\mu M$, pH 6.6–7.4; incubated at 37° C for 5 days; yield 12–32 arbitrary units/ml.[2]

Isolation. Activity of culture filtrate adsorbed on activated carbon and eluted with acid aqueous *n*-butanol; the eluate adjusted to pH 7.0, saturated with sodium chloride and extracted with *n*-butanol; the butanol extract shaken with water at pH 2.0, the aqueous phase separated and adjusted to pH 5.0.[3]

Purification. Crude ayfivin precipitated with picric acid, decomposed with ethanolic HCl; the hydrochloride further purified by countercurrent distribution.[3]

CHEMISTRY

Constitution. Polypeptide containing aspartic acid, glutamic acid, lysine, ornithine, histidine, cystine, phenylalanine and leucine and/or isoleucine. By analysis, C 48.8, H 7.3, N 14.6, S 1.5, Cl 7.9, amino N 2.2; *d*-α-amino N 14%.[3]

Properties. Soluble in water, ethanol; sparingly soluble in acetone; insoluble in ether; dialyzed through cellophane; no ultraviolet spectrum.[3]

Reactions. A weak base, isoelectric point at pH 7.1; stable at pH 2–10; inactivated in alkali or acid at 100° C; precipitated by picric, picrolonic and trichloroacetic acid, by Reinecke's salt, mercuric chloride, ammonium sulfate or sodium chloride; not precipitated by benzoic or salicylic acid; positive ninhydrin; negative ferric chloride, alkaline nitroprusside, Sakaguchi, Adamkiewicz; not inactivated by pepsin or trypsin; inactivated by copper.[3]

BACTERIOLOGY

Assay. Cylinder plate with *M. phlei* and *C. xerosis* [1, 2]; arbitrary unit of crude ayfivin hydrochloride, 5 units/mg; purified ayfivin hydrochloride 40 units/mg.[3]

Spectrum in vitro. Inhibition concentration (units/ml) [4]:

Staph. aureus	0.125–>1.0	*Cl. welchii*	>1.0
Staph. albus	1.0	*Cl. septicum*	>1.0
St. pyogenes ABDG	0.015–>1.0	*Cl. edematiens*	>1.0
St. fecalis	>1.0	*Cl. novyi*	>1.0
St. viridans	0.25 – 1.0	*B. anthracis*	>1.0
D. pneumoniae	0.031– 0.5	*Myco. phlei*	>1.0
Micro. lysodeikticus	0.031	*Myco. tuberc. bov.*	>2.0
Micro. tetragenus	1.0	*Myco. tuberc. hom.*	0.5–72
Micrococcus sp.	0.015	*Myco. tuberc.* BCG	0.5
N. gonorrheae	0.25	*E. coli*	>1.0
N. intracellularis	0.5– 1.0	*Past. pestis*	>1.0
C. diphtheriae	0.063–>1.0	*Pasteurella* sp.	>1.0
C. xerosis	0.008	*S. typhi*	>1.0
H. influenzae	>1.0	*S. enteriditis*	>1.0
H. parainfluenzae	>1.0	*Ps. aeruginosa*	>1.0
V. comma	>1.0	*Proteus* sp.	>1.0

Factors affecting activity. Little effect on activity by serum.[4]

Resistance. No resistance developed with *Staph. aureus;* some resistance with *M. tuberculosis.*[4]

Spectrum in vivo. In mice experimentally infected with *St. pyogenes,* 150 units administered intravenously over 2 days gave complete protection; with *Staph. aureus,* 160 units over 4 days gave complete protection up to 7 days and partial protection thereafter.[4]

PHARMACOLOGY

Toxicity. In mice, MLD intravenously 2.5–7.5 mg (30 units/mg); no evidence of chronic toxicity by repeated sublethal doses; evidence of kidney damage but not related to number of units administered.[4]

Nonhemolytic; leucocytes unaffected at 1:1,000.[4]

Blood levels. In rabbits, 960 units subcutaneously, produced 2.0 units/ml in the serum at one-half hour after administration, 0.25 unit/ml after 3 hours.[4]

Excretion. In mice, activity detected in the urine following subcutaneous administration.[4]

Absorption. No activity detected in the urine following oral administration.[4]

Physiology. In anaesthetized cats, 100–200 mg caused a fall in blood pressure; at 1:5,000, little effect on the isolated guinea pig uterus.[4]

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BACILIPIN

First described by Newton in 1949; derived from *Bacillus subtilis;* BACILIPIN A and B have been described.

PRODUCTION

Fermentation. Submerged growth in aerated vessels on Czapek-Dox medium containing neutral corn steep liquor 0.5% and glucose 0.5%; incubated at 34° C for 12 hours. (By variation in air rate, bacilipin A and B, BACILYSIN or another unstable antibiotic is produced.)

Isolation. Culture filtrate at pH 5.0 extracted with amyl acetate.

Purification. Amyl acetate solution adsorbed on alumina at pH 9.0; activity eluted with phosphate buffer saturated with acetone, extracted into ether; bacilipin A and B separated by countercurrent distribution between ether and buffer; yield from 200 liters, 110 mg of bacilipin A and 60 mg of bacilipin B.

CHEMISTRY

Constitution. By analysis of barium salt: bacilipin A, C 42.6, H 6.3, N 2.5, Ba 24.6, equivalent weight 278; bacilipin B, C 52.5, H 6.75, N 2.09, Ba 21.6, equivalent weight 317.5.

Properties. Soluble in amyl acetate, benzene, chloroform, ether; slightly soluble in water; insoluble in petroleum ether; bacilipin salts soluble in water and absolute alcohol.

Reactions. Activity lost on drying; free acid unstable; salts stable in solution from pH 2.0–10.0; heat-labile; nondigested by trypsin; negative Molisch, 2,4-dinitrophenylhydrazine, ammoniacal silver nitrate; readily catalytically hydrogenated.

BACTERIOLOGY

Assay. Cylinder plate with *M. phlei* or *Staph. aureus.*

Spectrum in vitro. Active against *M. phlei*, *Staph. aureus*, *C. diphtheriae* *gravis*, *C. xerosis*, *B. anthracis*, *S. enteriditis*, *S. typhi* and *E. coli;* unaffected by serum.

Mode of action. Bacteriostatic.

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BACILLIN

First described by Foster and Woodruff in 1946[1]; derived from *Bacillus subtilis*.

PRODUCTION

Fermentation. Surface growth on medium containing glucose, alanine, salts and 20 ppm $MnSO_4 \cdot 4H_2O$; yield 48 *E. coli* units/ml.

Isolation. Activity of culture filtrate (A) adsorbed on activated carbon, eluted with 90% ethanol, eluate evaporated in vacuo to dryness[1, 3]; (B) adsorbed on cationic resin, eluted with dilute aqueous pyridine.[3]

CHEMISTRY

Insoluble in organic solvents; heat-stable; inactivated by H_2S.[1, 3]

BACTERIOLOGY

Assay. Serial dilution by agar-streak; cylinder-plate method with *E. coli;* most potent preparation 50 units/mg.[1]

Spectrum in vitro. Inhibition dilution[1]:

E. coli	160–240	*D. pneumoniae* I	480
Eb. typhi	240	*D. pneumoniae* II	120
S. paratyphi	1,280	*S. aureus*	320
S. schottmuelleri	80	*S. albus*	320
Pasteurella sp.	320	Streptococcus (on blood)	960
Micro. conglomeratus	160	*Myco. tuberc.* (on synthetic)	640

Factors affecting activity. Activity reduced by brain heart media, by rabbit blood,[1] by antibacillin.[2]

Spectrum in vivo. No protection for mice infected with *Staph. aureus* and *D. pneumoniae*.[1]

PHARMACOLOGY

In mice, 20 mg administered intraperitoneally was highest tolerated dose.[1]

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BACILLOMYCIN

First described by Landy, Warren, Rosenman and Colio in 1948; derived from *Bacillus subtilis*.

PRODUCTION

Fermentation. Surface and submerged culture in shaker flasks on medium containing g/l glucose 20, *l*-glutamic acid 5, $MgSO_4$ 0.5, KCl 0.5, KH_2PO_4 1.0, $Fe_2(SO_4)_3 \cdot 6H_2O$ 0.00015, $MnSO_4 \cdot H_2O$ 0.005, $CuSO_4 \cdot 5H_2O$ 0.00016, pH 6.0, at 24–28° C; maximal yields at 2–3 days in submerged culture, 5–6 days in surface culture at final pH 7.0–7.6.

Isolation. Filtered fermented liquor acidified to pH 2.5 with HCl, filtered, precipitate extracted with ethanol, washed with ether, dried in vacuo over phosphorus pentoxide (the ether wash removes another antibiotic which is active against gram-positive bacteria); yield approximately 0.3 g/l representing 35–50% recovery.

CHEMISTRY

Soluble in methanol, ethanol, *n*-butanol, acetone; insoluble in ether; precipitated by ammonium sulfate; adsorbed by activated carbon, but eluted with difficulty; non-dialyzable through cellophane membranes; not destroyed by trypsin, pepsin; stable in dry form; solutions at pH 3.0–9.0 are thermostable.

BACTERIOLOGY

Assay. Cylinder-plate method on Sabouraud's maltose agar using spore suspension of *Trichophyton mentagrophytes* incubated at 30° C for 72 hours; unit equivalent to 0.1 mg of arbitrary standard bacillomycin producing a 20–25 mm zone of inhibition.

Spectrum in vitro:

Antibacterial activity is negligible.

Fungistatic activity, inhibition concentration (mg/ml)[1]:

Epidermophyton floccosum	0.025
Microsporon audouini	0.025
Microsporon gypseum	0.025
Trichophyton mentagrophytes	0.025
Trichophyton rubrum	0.025
Trichophyton schoenleini	0.025
Blastomyces dermatitidis (mycelial)	0.010
Blastomyces dermatitidis (yeast)	0.0025
Candida albicans	0.05
Coccidiodes immitis	0.025

Torula histolytica	0.05
Histoplasma capsulatum	0.025
Hormodendrum pedrosoi	>0.5
Nocardia asteroides	>0.5
Monosporium apiospermum	0.025
Phialophora verrucosa	>0.5
Sporotrichum schenkii	0.05
Blastomyces braziliensis	0.025

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BACILLOSPORIN *See* POLYMYXIN

BACILYSIN

First described by Newton in 1949; derived from *Bacillus subtilis.*

PRODUCTION

Fermentation. Submerged growth in aerated vessels on Czapek-Dox medium containing neutral corn steep liquor 0.5% and glucose 0.5%; incubated at 34° C for 12 hours. (By variation in air rate bacilysin, BACILIPIN A and B or another unstable antibiotic is produced.)

Isolation. Culture liquor clarified at pH 3.0, adjusted to pH 2.0; activity adsorbed on activated carbon, eluted with ethanol-phosphate buffer.

Purification. Activity adsorbed on alumina column at pH 5.0, eluted with 25% ethanol, the eluate distilled *in vacuo* and the residue freeze-dried.

CHEMISTRY

Constitution. Neutral peptide containing alanine, tyrosine, leucine and phenylalanine, with nitrogen and sulfur.

Properties. Insoluble in dry organic solvents except methanol, soluble in water.

Reactions. Stable at pH 1.4–12.0; heat-stable; not precipitated by ammonium sulfate, basic lead acetate and by picric, phosphotungstic or trichloroacetic acid; negative Molisch; positive ninhydrin, Pauly; inactivated by trypsin, tissue enzymes and by hydrogen sulfide.

BACTERIOLOGY

Assay. Cylinder plate with *M. phlei* and *Staph. aureus.*

Spectrum in vitro. Active against *Staph. aureus* and *C. xerosis;* less active against *M. phlei* and *E. coli.*

Mode of action. Bactericidal.

Resistance. Resistant colonies inside zone of assay plate.

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BACITRACIN

First described by Johnson, Anker and Meleney in 1945 [1]; derived from *Bacillus subtilis.*

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l *l*-glutamic acid 5, KH_2PO_4 0.5, K_2HPO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.2, $MnSO_4 \cdot 4H_2O$ 0.01, NaCl 0.01, $FeSO_4 \cdot 7H_2O$ 0.01, $CuSO_4 \cdot 7H_2O$ 0.01, $CaH_4(PO_4)_2$ sat. sol. 2 ml, pH 6.8-7; incubated at 37° C for 72 hours, final pH 7.0–7.2; average yield 6 *St. hemolyticus* units/ml. (B) Soybean digest; average yield 12 units/ml.[2]

Isolation. Culture filtrate extracted with *n*-butanol; butanol extract concentrated *in vacuo* with addition of water.[2]

Purification. (A) Crude concentrate acidified with HCl to pH 3–4 and washed with *n*-butanol ether (peroxide-free); aqueous phase neutralized and freeze-dried; final product 10–20 units/mg. (B) Treatment with magnesium oxide to remove pigments. (C) Crude concentrate acidified with HCl to pH 4, solid salicylic acid added, the first precipitate discarded; the filtrate neutralized and additional solid salicylic acid added; the precipitate removed and dried, washed with ether (peroxide-free) and dried *in vacuo;* the precipitate dissolved in dilute HCl.[2]

CHEMISTRY

Constitution. A polypeptide; contains lysine, ornithine, *l*-histidine, partially racemic *l*-leucine, *l*-cystine, *l*-glutamic acid, *dl*-phenylalanine, *dl*-aspartic acid, and partially racemic *d*-isoleucine.[3]

Properties. White, highly hygroscopic powder [3]; soluble in methanol, ethanol, isopropanol, *n*-butanol and cyclohexanol; slightly soluble in cyclohexanone; insoluble in ether, chloroform, benzene, acetone and ethyl acetate; diffused readily through a nitrocellulose membrane; $[\alpha]_D^{23} = +5 \pm 2.5$ (1% in 0.02*N* HCl).[2]

Reactions. Stable in neutral and slightly acid solution at 0–5° C, inactivated by 2 weeks' incubation at 35–37° C; inactivated by hydrogen peroxide, BAL and sodium thiosulfate; precipitated by salts of heavy metals, by trichloroacetic, tannic, azobenzene-*p*-sulfonic, benzoic, furoic and salicylic acids, by high concentrations of sodium chloride, by acetone, ammonium rhodanilate, Reinecke's salt and molybdic acid; adsorbed on activated carbon, Lloyd's reagent and aluminum oxide; negative biuret, Sakaguchi and Folin-Marenzi [2]; not digested by pepsin or trypsin.[1]

BACTERIOLOGY

Assay. Serial dilution with *St. hemolyticus* [1, 4]; cylinder-plate and turbidimetric with *Staph. aureus* [5]; cylinder-plate with *St. hemolyticus* for bacitracin in serum, urine and saliva [6]; maximum activity 66 units/mg.[3]

Spectrum in vitro. Inhibition concentration (units/ml) [1, 7, 8, 9]:

St. hem. β (ABCFG)	0.025–0.005	*B. anthracis*	4–12.5
St. hem. β (D)	3–0.008	*Flavobacterium*	0.0025
St. non-hem.	3–0.025	*H. influenzae* B	0.63
Pneumococcus	0.1 –0.902	*Cl. welchii*	0.025– 0.002
Staphylococcus (coag. +)	5–0.05	*Cl. septicum*	0.01 – 0.002
Micrococci (aerobic)	5–0.008	*Cl. sordellii*	0.01 – 0.005
C. xerose	0.005–0.003	*Cl. novyi*	0.01
C. diphtheriae	0.015–0.004	*Cl. tetani*	0.01 – 0.006
N. meningitidis	0.01	*Cl. histolyticum*	0.025– 0.004
N. gonorrheae	0.006	Diphtheroids	0.003

St. hem. (anaerobic)	0.01 –0.001
St. non-hem. (anaerobic)	0.1 –0.005
Micrococci (anaerobic)	0.5 –0.005
Actinomyces israeli	0.075–0.005
Trep. pallidum	0.004

Not inhibited at 50 units/ml: *B. subtilis*, *E. coli*, *A. aerogenes*, *A. cloacae*, *Proteus*, *Ps. aeruginosa*, *B. alkaligenes*, *S. typhosa*, *Sh. alkalescens*, *Monilia albicans*, *Cryptococcus hominis*, *Nocardia asteroides*.[7]

Synergism in vitro. Bacitracin was synergistic with penicillin against several strains of α and β hemolytic streptococci.[10]

Mode of action. Rate of lethal action of bacitracin increased progressively against *Trep. pallidum* up to the highest concentration used.[9]

Experimental infections in animals. In mice infected with hemolytic streptococcus, 1–2 units intraperitoneally allowed 80% survival; 1–2 units subcutaneously allowed only 30–40% survival, but the same dose repeated every 3 hours for 36 hours resulted in 80–90% survival.[1] In guinea pigs infected with *Cl. welchii* or with *Cl. septicum*, 50–100 units subcutaneously every 3 hours for 36 hours allowed 80% survival [1]; with *Cl. welchii*, 3 units/g administered 2 hours after infection resulted in 98% protection.[11] In rabbits, infected with *Trep. pallidum*, 1,200 units/kg intramuscularly was treponematicidal, 2,300 units/kg per day for 4 days allowed 50 per cent protection.[9, 12, 13] In dogs infected with staphylococcal meningitis, 4,000–10,000 units intracisternally supplemented by 10,000–50,000 units intramuscularly, administered 3 hours after infection, gave full protection.[14] In dogs, baci-

tracin administered orally, 2,000–10,000 mg/kg/day, greatly reduced fecal streptococci and spore-forming anaerobes.[15]

Synergism in vivo. In rabbits experimentally infected with *T. pallidum*, one-seventh of the 50% curative bacitracin dose administered in conjunction with one-fortieth of the 50% curative penicillin dose, were completely curative, or a sevenfold increase in effectiveness.[13]

Veterinary. Bacitracin, at 20,000–100,000 units per quarter, was effective against bovine mastitis caused by *St. agalactiae*.[16, 17]

Clinical. In surgical infections, 0.1–5.0 ml of a 10–100 unit/ml solution or as an ointment, gave a favorable response in 88% of a large number of cases [18]; 2,000–52,000 units administered parenterally every 6 hours gave a good response.[19] In superficial mixed pyogenic infections, a bacitracin ointment, 480 units/g, was used effectively.[20] Bacitracin systemically and topically gave a 65.6% favorable response in a large number of miscellaneous infections, including a large proportion of infections resistant to therapy with penicillin, streptomycin and sulfonamides.[7] In eye infections, bacitracin applied as a fine powder or in saline at 1,000–5,000 unit/ml penetrated injured or inflamed corneas; good results were obtained when used for acute infections, less effective against chronic infections.[21] Bacitracin in the form of 1,000 unit lozenges was effective in the therapy of mild, acute, and chronic pharyngeal and tonsillar infections, but was not effective with severe infections; in solution at 100 units/ml or greater, bacitracin was effective against mastoid infection, less effective with external otitis and chronic suppurative mastoiditis.[22]

PHARMACOLOGY

Toxicity. In mice, LD 50 mg/kg[23]:

intraperitoneal	200–650	(7,500–15,000 units)
intravenous	360	(7,500 units)
subcutaneous	1,300–2,500	(31,000–52,000 units)
oral	3,750	tolerated (131,000 units)

In rats, LD 50 mg/kg, intraperitoneally, was 190 (6,700 units).[23] No chronic toxicity in rats and mice by repeated sublethal doses.[23] In dogs and monkeys, 1,000–1,500 units/kg 3 times a day for 23 days intramuscularly, of a crude bacitracin concentrate produced no blood pathology and only small areas of induration and necrosis at the site of injection.[25] In rabbits, intradermal injection and installation into the conjunctival sac caused only slight irritation [25]; tolerated when applied to the eye, did not penetrate through normal cornea, did penetrate through traumatized cornea.[24] Clinically, sensitivity reactions to bacitracin topically were rare, even after protracted therapy.[19]

Nephrotoxicity. After prolonged intramuscular administration of crude bacitracin, glycosuria and albuminuria observed in the monkey but not in *the dog [24]; LD 50 bacitracin dose in mice caused kidney tubule necrosis [23];* renal damage in mice, minimal in rats [23]; bacitracin nephrotoxicity in mice variable, resistant strains showing no toxicity.[26] Clinically, toxic kidney reactions were noted [18]; minimized by the use of low-toxicity bacitracin, *i.e.*, LD 50 = >500 units per 20 g mouse.[7, 27]

Blood levels, Excretion. In dogs [24, 28]:

	Dose units/kg	Hours after dose	Blood units/ml	Urine, units/ml at 7 hrs.
subcutaneous	3,000	3–4	3	20
intramuscular	3,000	1–2	1.5	92
intravenous	3,000	5	3–7	72

Spinal fluid concentrations negligible.[28] In rabbits, 10.8 units/kg intravenously, produced 0.5 unit/ml at 5 hours after administration.[4]

In humans, bacitracin at 15–150 units/kg intramuscularly produced blood levels after 4 hours of 0.015–0.14 unit/ml.[4]

Renal clearance of bacitracin averaged, in rabbits 4.1 ml/min., in humans 159 ml/min.[4, 29]

Absorption, Diffusion. In dogs with oral administration, 3,000–6,000 units/kg, no detectable bacitracin appeared in the blood and urine, and less than 5% recovered in the stool [28]; with 10,000 units/kg/day, blood levels up to 0.085 unit/ml in the blood and 0.22 unit/ml in the urine were obtained one hour after administration [14]; bacitracin persisted in the feces for 3 days after cessation of dosage.[15]

Upon intravenous administration, bacitracin was found distributed through all the organs, tissues and body fluids, except for very low concentrations in the brain and cerebrospinal fluid.[3]

Physiology. In dogs, intravenously, bacitracin caused a transient fall in blood pressure followed by a transient rise. With guinea pig ileum, 100 mg or 3,000 units was equivalent to 3γ of histamine diphosphate. With frog heart, high concentrations of bacitracin caused slowing and diminished contraction.[23]

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BIFORMIN

First described by Robbins, Kavanagh and Hervey in 1947; derived from *Polyporus biformis;* also see BIFORMINIC ACID (acid form of biformin).

PRODUCTION

Fermentation. Surface growth in medium containing g/l $NaNO_3$ 3.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $Fe_2(SO_4)_3$ 0.01, dextrose 40.0, corn steep liquor solids 5.0 with beech wood shavings; fermentation time 2 weeks or more at 25° C; mycelium may be drained off fermented liquor and refilled with fresh medium twenty times with no decrease in production; yield, 256 dilution units (*Staph. aureus*) of which about 10% is biforminic acid.

Isolation. Fermented liquor boiled 10 min. at pH 6.5 (thereby increasing yield 4–8 times), extracted twice with chloroform, evaporated with addition of water to turbid brown aqueous concentrate and filtered yields product with 900–1,750 dilution units per mg.

Purification. Precipitated with $AgNO_3$ and HNO_3, filtered, Ag removed from yellow precipitate by KI and HCl, filtrate neutralized, extracted with ether, evaporated to an aqueous concentrate, filtered; yields product with 3,600 dilution units per mg.

CHEMISTRY

Soluble in water, ether, chloroform, methyl isobutyl ketone, alcohol; stability unaffected by boiling in 0.01*N* acid or alkali; stable in dilute solution, unstable in concentrated solution; destroyed by drying; no color with KCN, no iodine from cold HI, no color with ferric chloride.

BACTERIOLOGY

Spectrum in vitro. Inhibition (γ/ml):

B. mycoides	5–13	*Ps. aeruginosa*	32–50
B. subtilis	0.04	*Myco. phlei*	0.6–2.5
E. coli	1.6	*Myco. smegmatis*	3–3.5
K. pneumoniae	0.6 –1.6	*Myco. tuberculosis*	0.56
Photo. fischeri	0.02	*Staph. aureus*	0.3–0.8

Biformin shows anti-fungal activity.

Factors affecting activity. Serum has little to no effect using *Staph. aureus;* 5% defibrinated rabbit blood reduced activity 90%.

Resistance. No resistance developed by *Staph. aureus.*

Spectrum in vivo. No mouse protection against *Staph. aureus* and *Myco. tuberculosis.*

PHARMACOLOGY

MLD mouse, mg/kg: intravenous 18.8, intraperitoneal 37.5, subcutaneous 77.15. Severe dermatitis upon contact in humans.

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BIFORMINIC ACID

First described by Robbins, Kavanagh and Hervey in 1947; derived from *Polyporus biformis;* also see BIFORMIN (neutral form of biforminic acid).

PRODUCTION

Fermentation. Like biformin. Yield about 10% of total antibacterial activity (256 *Staph. aureus* dilution units).

Isolation. Following extraction of biformin, reextracted at pH 2 with chloroform (yield not increased by boiling).

Purification. Chloroform solution extracted with one per cent sodium bicarbonate solution, washed with ether, acidified to pH 2 and extracted three times with equal volume ether; ether extracts washed several times with water, reextracted from ether with one per cent sodium bicarbonate.

CHEMISTRY

Impure biforminic acid gives brown color with ferric chloride.

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (γ/ml):

B. mycoides	3.4	*Myco. phlei*	0.8
B. subtilis	0.2	*Myco. smegmatis*	6.8
E. coli	3.4	*Staph. aureus*	0.7
K. pneumoniae	6.8	*Ps. aeruginosa*	220

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Robbins, Wm. J., Kavanagh, Frederick, and Hervey, Annette, "Antibiotics from Basidiomycetes. II. Polyporus biformis," *Proc. Nat. Acad. Sci.*, **33**, 176 (1947).

BIOCERIN

Discovered by Goodlow, Johnson and Shafer in 1947 [1]; named by Johnson, West, Jones and Long in 1949 [2]; derived from *Bacillus cereus.*

PRODUCTION

Fermentation. Surface growth on medium containing g/l glucose 2.0, $NH_4H_2PO_4$ 1.0, NaCl 2.0, $MgSO_4$ 1.0,K_2HPO_4 1.0, methionine 0.005, agar 5.0; incubated at 37° C for 48 hours.[2]

Isolation. Culture liquor extracted with ether, and the ether extract allowed to evaporate to a brownish-yellow oily residue.[2]

CHEMISTRY

Soluble in 95% ethanol, acetone, ether and chloroform; insoluble in water.[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration of crude biocerin (mg per ml) [2]:

Staph. aureus	0.05–0.025
S. typhosa	0.5 –0.1
S. paratyphi A	1.0 –0.5
S. paratyphi B	1.0 –0.5
Coryne. diphtheriae	0.5 –0.1
B. anthracis	0.5 –0.1
B. subtilis	0.5 –0.1
Sarcina lutea	1.0 –0.5
E. coli	0.5 –0.1
Brucella suis	0.5 –0.1
Aero. aerogenes	0.5 –0.1
Neis. catarrhalis	0.5 –0.1

Factors affecting activity. Activity decreased by 10–20% rabbit serum.[2]

PHARMACOLOGY

Intraperitoneal injection of 20 mg crude biocerin in mineral oil in mice produced no fatalities at 5 days and no toxic symptoms.[2]

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BORRELIDIN

First described by Berger and Goldberg in 1949 [1]; produced by *Streptomyces rochei*, named from its antiborrelia activity. Borrelidin-like substances can be detected in impure penicillin, in "clarase" and in corn steep liquor.[1,2,3,4]

PRODUCTION

Fermentation. Surface and submerged growth on medium containing soybean meal, glucose and phosphate.[1]

Isolation. Culture liquor extracted with butyl acetate, the extract evaporated, the residue dissolved in solvent, treated with bentonite clay, extracted from ether with alkali.[3]

Purification. Crude borrelidin dissolved in benzene, concentrated and crystallized; recrystallized from benzene.[3]

CHEMISTRY

Constitution. Proposed $C_{28}H_{43}O_6N$; an acid.

Properties. M.p. 145–146° C; $[\alpha]^{27}_{D} = -28°$ in ethanol; ultraviolet maximum at 256 mμ, E 1%/1 cm = 550.[3]

BACTERIOLOGY

Spectrum in vivo. In mice experimentally infected with *Borrelia*, the following curative doses, administered subcutaneously, were obtained in mg/kg: *Borrelia* (Tick strain) 0.815, *B. novyi* 3.25, *B. obermeieri* 3.6.[4]

Resistance. By serial passage, a resistant strain of *B. obermeieri* was obtained.[4]

Synergism. Enhancement of activity of penicillin G by small doses of borrelidin against syphilis in rabbits, beta-hemolytic streptococcus infection and meningococcus endotoxin in mice; no enhancement in infections with *D. pneumoniae*, *E. typhi* or *S. schottmuelleri*.[4, 5, 6]

PHARMACOLOGY

Toxicity. LD 50 mg/kg, in mice subcutaneously 74.7, intravenously 39.0; in rat subcutaneously 1.78; in rabbit intravenously 2.37; in chick intramuscularly 74.2 [4, 7]; irritating to tissues of animals and skin of humans.[7]

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CANDIDULIN

First described by Stansly and Anenenko in 1949[1,2]; derived from *Aspergillus candidus.*

PRODUCTION

Fermentation. Surface growth on medium containing glucose, sodium nitrate and potassium phosphate.[1] Submerged growth in shaker flasks on medium containing glucose 0.5%, glycerol 1.5%, $NaNO_3$ 0.1%, KH_2PO_4 0.05% with either agar 2% or asparagine 0.2%; incubated for 7 days at 25° C; yield 64 *M. ranae* units/ml; no activity produced on media containing corn steep liquor, yeast extract, soybean flour.[2]

Isolation. Culture filtrate extracted with ether or chloroform, the extract washed with water, dried and evaporated *in vacuo* to dryness.[2]

Purification. (A) Residue taken up in chloroform, impurities removed by passage through a magnesium sulfate column, then activity adsorbed by an activated alumina column. (B) Residue dissolved in hot *n*-hexane, cooled and crystallized; yield 5 mg/l.[2]

CHEMISTRY

Constitution. Proposed $C_{11}H_{15}NO_3$, M.W. 209.13; calculated C 63.1, H 7.2, N 6.7; found C 63.47, 63.53, H 7.3, 6.9, N 6.6; M.W. 232 (osmotic); no acid or basic groups.[2]

Properties. Long white needles, m.p. 88–89° C; refractive index 1.525 ± 0.003; soluble in methanol, ethanol, acetone, ether; soluble mg/ml in chloroform >6, benzene 5, carbon tetrachloride 4, *n*-hexane 0.075, boiling *n*-hexane *ca.* 1.0, water 5; $[\alpha]_D^{24} = +15° \pm 2$ (1% in chloroform); no characteristic ultraviolet spectrum.[2]

Reactions. Negative ferric chloride, ninhydrin, 2,4-dinitrophenylhydrazine, 3,5-dinitrobenzylchloride, xanthhydrol, HgO; inactivated by bromine in carbon tetrachloride; thermostable; alkali-labile.[2]

BACTERIOLOGY

Assay. Agar streak method with *M. ranae.*[2]

Spectrum in vitro. Inhibition concentration (γ/ml.)[2]

Staph. aureus	320	*Ps. aeruginosa*	640
Sarcina lutea	320	*E. coli*	160
St. hemolyticus	160	*Br. abortus*	20
B. polymyxa	160	*K. pneumoniae*	10
B. subtilis	10	*Streptomyces griseus*	320
Myco. ranae	2.5	*Asp. fumigatus*	80
Myco. smegmatis	2.5	*Pen. puberulum*	40

Mycobacterium sp. 1.25

Factors affecting activity. Inactivated by whole blood.[2]

Spectrum in vivo. No protection against experimental infections with *Myco. tuberculosis* in mice.[2]

PHARMACOLOGY

Toxicity. In mice, administered subcutaneously, the LD 50 was *ca.* 250 mg/kg.

Blood levels, excretion. Not detected in blood or urine.[2]

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CASSIC ACID

Antibiotic activity of *Cassia reticulata* first observed by natives of Costa Rica who used it orally for the therapy of chronic gonorrhea; first described by Robbins, Kavanagh and Thayer in 1947.

PRODUCTION

Isolation. Leaves washed in boiling water, washings discarded; boiled with water and dilute sodium carbonate, cooled aqueous extracts acidified with HCl to pH 3.2, extracted with methyl isobutyl ketone; extract concentrated in vacuo to an oily residue.

Purification. (A) Crude material dissolved in acetone, water added and activity removed by ether extraction; ether solution extracted with dilute sodium bicarbonate; acidification of bicarbonate extract precipitated crystalline cassic acid. (B) Crude material dissolved in alcohol, petroleum ether added, filtered and precipitate discarded; filtrate extracted with ether, ether extract evaporated to dryness; residue dissolved in acetone, excess petroleum ether added and precipitate discarded; filtrate concentrated, filtered free of precipitate and evaporated to dryness; residue dissolved in ether, extracted with dilute sodium bicarbonate; bicarbonate extract acidified and extracted with ether; ether extract evaporated to dryness; yield 58 mg per 50 g.

CHEMISTRY

Constitution. No S, N, Cl, Br or I.

Properties. Yellow needles; m.p. 330° C (dec. and subl.); weakly acid; slightly soluble in alcohol, ether, chloroform, methyl isobutyl ketone, water; nearly insoluble in petroleum ether; yellow in acid, red in alkaline solution.

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus* and *E. coli.*

Spectrum in vitro. Inhibition concentration of crystalline sodium salt (γ/ml):

Staph. aureus	8	*N. gonorrheae*	8
B. subtilis	8	*E. coli*	>250
B. mycoides	4	*K. pneumoniae*	>250
Myco. phlei	8	*Ps. aeruginosa*	>250
Myco. smegmatis	32		

PHARMACOLOGY

In mice and rabbits, low toxicity upon oral administration; activity detected in urine.

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CHETOMIN

First observed by Waksman and Horning in 1943[1]; isolated and named chaetomin by Waksman *et al.* in 1944[2,3,4]; spelling simplified to chetomin in 1949[5]; derived from *Chaetomium cochliodes*[3]; also from *C. funiculum, C. elatum,*[3] *C. spirale, Verticillium cinnabarinum* (*Acrostalagmus cinnabarinus*).[6]

PRODUCTION

Fermentation. By *C. cochliodes* in surface and submerged culture on medium containing g/l glucose 30, corn steep solids 5 ml, KNO_3 2, KCl 0.5, KH_2PO_4 0.5, $MgSO_4$ 0.5, $CaCO_3$ added, pH 7.0; incubated at 28° C for 10 days surface and 10–14 days submerged; yield 750 *B. subtilis* units/ml in culture filtrate of surface fermentation, 2,000 *B. subtilis* units/ml in culture filtrate of submerged fermentation[3]; yield 200 *Staph. aureus* units/ml in culture filtrate, 10,000 *Staph. aureus* units/ml from mycelium.[4]

Isolation. *From culture filtrate:* extracted with ethyl acetate, the extract concentrated in vacuo, washed with sodium bicarbonate solution, with sodium carbonate solution and with water until neutral; evaporated in vacuo to a brown, gummy residue; yield 10 mg/l.

From mycelium: extracted with acetone, the extract concentrated in vacuo and extracted with ethyl acetate (sodium chloride added to prevent emulsions); the ethyl acetate extract washed with sodium bicarbonate solution, with sodium carbonate solution and with water until neutral; evaporated in vacuo to dryness; yield of crude chetomin 100–150 mg per weight of mycelium equivalent to one liter of culture liquor.[4]

Chetomin from the culture filtrate and from the mycelium are considered identical on basis of chemical and bacteriological properties.[4]

Purification. (A) Crude chetomin dissolved in ethyl acetate, precipitated with cold petroleum ether, the precipitate dissolved in benzene and passed through an alumina column; the column washed with benzene, eluted with 10% absolute methanol in benzene, eluates evaporated in vacuo to dryness; the residue dissolved in ethyl acetate, cold petroleum ether added, precipitating chetomin; precipitate dried in vacuo; yield about 90% of activity of acetone extract of mycelium.[4]

(B) Crude chetomin dissolved in benzene, activity adsorbed on an activated carbon column; the column washed with benzene, eluted with 1% anhydrous methanol in benzene; eluates evaporated to dryness; the residue dissolved in chloroform, chetomin precipitated with cold petroleum ether.[5]

CHEMISTRY

Constitution. Proposed $C_{16}H_{17}O_4N_3S_2$; M.W. 310 (ethylene bromide).[5]

Properties. M.p. 218–220° C (decomp.),[4] 215° C[5]; $[\alpha]_D^{22}$ 360° (1% in chloroform); ultraviolet maximum at 287 mμ [5]; soluble in acetone, ethyl acetate, chloroform, benzene, dioxane, pyridine; less soluble in ether, methanol, ethanol; insoluble in water, petroleum ether.[4]

Reactions. No free acid or basic groups; stable in aqueous suspension with gum acacia; stable in dilute acid, unstable in alkali; not precipitated by picric acid, mercuric chloride; no amino nitrogen; positive indole, Hopkins-Cole, dimethylaminobenzaldehyde; negative biuret, Millon, dimethylaminobenzaldehyde; no reaction with diazomethane; not inactivated by penicillinase; inactivated by oxidizing agents, alkalies and hot acids [4, 5]; sulfur is not released by heating with sodium plumbite or aqueous alkali[4]; forms lead sulfide with sodium plumbite.[5]

BACTERIOLOGY

Assay. Serial dilution with *B. subtilis*,[3] *Staph. aureus* [4]; agar streak method using chetomin in suspension in 5% aqueous gum acacia with *Staph. aureus*, *B. subtilis*, *B. mycoides*, *Sarcina lutea*.[5]

Spectrum in vitro. Inhibition dilution of crude chetomin [3]:

B. subtilis	30,000–175,000	*Sarcina lutea*	200,000
B. mycoides	40,000	*Ph. michiganensis*	200,000
B. cereus	1,500	*Ph. prunei*	200,000
B. mesentericus	60,000	*Ph. phaseoli*	200,000
B. megatherium	60,000	*Clos. butyricum*	6,000
Staph. aureus	500,000	*E. coli*	<2,000
Myco. phlei	20,000	*S. marcescens*	<2,000
Myco. tuberc. av.	3,000– 17,500	*A. aerogenes*	<2,000
Myco. tub. human	3,000– 17,500	*Pr. vulgaris*	<2,000
List. monocytogenes	20,000	*Salmonella*	<2,000
Ps. fluorescens	1,000	*Ps. aeruginosa*	<2,000

Inhibition dilution with purified chetomin[4]:

Staph. aureus	500,000	*B. subtilis*	500,000
B. mycoides	100,000	*Sarcina lutea*	1,000,000

Spectrum in vivo. No protection in experimental infections in animals against organisms susceptible in vitro to chetomin.[3]

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CHLORAMPHENICOL

First described by Ehrlich, Bartz, Smith and Joslyn in 1947[1]; independently described by Gottlieb, Bhattacharyya, Anderson and Carter in 1948[2]; derived from *Streptomyces venezuelae*[3, 4] and by synthesis.[5, 6, 7, 8] "CHLOROMYCETIN" is the trade mark for chloramphenicol.

PRODUCTION

Fermentation. (A) Submerged growth in shaker flasks, 30 liter glass fermenter and 200 gal. rotary aluminum drum, on medium containing maltose 1.0%, casamino acid 0.5%, distiller's solubles 0.5%, NaCl 0.5%; yield γ/ml, shaker flasks 55, 30-l glass fermenter 85, rotary aluminum drum 49.[1] (B) Submerged growth in shaker flasks on medium containing g/l soybean meal 10, "cerelose" 10, NaCl 5.0, "Curbay BG" 0.5, $CaCO_3$ 1.0; incubated 4–5 days; yield 260 streptomycin units/ml or 80 γ/ml.[2] (C) Submerged growth in shaker flasks and aerated tanks on medium containing glycerol 1%, hog stomach residue 0.5%, NaCl 0.5%, molasses 1.0; pH 6.7; incubated at 23–27° C for 3–6 days, final pH 5.9; yield 142 γ/ml in 3 days.[9]

Isolation. (A) Culture filtrate acidified and extracted with ethyl acetate, the extract evaporated in vivo to dryness; the residue extracted with ether.[1] (B) Culture filtrate extracted with ether, the ether extract evaporated to dryness.[2] (C) Culture filtrate extracted with ethyl acetate, the extract dried over anhydrous sodium sulfate, evaporated *in vacuo* to dryness, the residue extracted with ether.[10, 11] (D) Culture filtrate extracted with ethyl acetate, the extract concentrated and kerosene added, the mixture washed with dilute sulfuric acid, with dilute sodium bicarbonate and with distilled water, then dried over anhydrous sodium sulfate; the solution distilled *in vacuo* to a concentrate, chilled and allowed to crystallize; crystals washed with petroleum ether.[10] (E) Activity of culture filtrate adsorbed on activated carbon, eluted with organic solvents.[11]

Purification. (A) Crude ether solution chromatographed over aluminum oxide, the eluate evaporated to dryness, the residue extracted with water, the water extract washed with petroleum ether, concentrated and allowed to crystallize; recrystallized from methylene dichloride, ethylene dichloride and ether-petroleum ether.[1, 10] (B) Crude residue washed with hot benzene, then recrystallized from benzene-methanol; chromatographed over alumina in chloroform, eluted with methanol; the eluate evaporated to dryness and recrystallized from chloroform-methanol; yield 62%.[2] (C) Crude crystals dissolved in ethylene dichloride, treated with activated carbon and recrystallized.[10]

Synthesis. From 1-phenyl-2-nitro-1,3-propanediol [5]; from *p*-nitroacetophenone [7]; synthesis intermediates by monohydroxymethylation of α-acylamidoacetophenone followed by catalytic hydrogenation.[6]

Large-scale production by fermentation and by synthesis.[8]

CHEMISTRY

Constitution. D(-)threo-*l*-*p*-nitrophenyl-2-dichloracetamido-1,3-propanediol.[12]

Properties. Colorless elongated plates or fine needles [1, 2, 10]; m.p. 149.7–150.7° C (cor.),[1, 10] 144–146° C [2]; soluble in water (2.5 mg/ml at 25° C), methanol, ethanol, butanol, propylene glycol (150.8 mg/ml), 70% aqueous propylene glycol (60.6 mg/ml), acetone, ethyl acetate; slightly soluble in chloroform, ether; slowly soluble in cold dilute alkali; insoluble in acid, benzene, petroleum ether, vegetable oils.[1, 2, 10, 13] Optical rotation $[\alpha]^{25}_{D} = -25.5°$ (ethyl acetate) [1, 10]; ultraviolet absorption maximum 278 mμ.[2, 10]

Reactions. Stable from pH 0.40–9.56 [11]; chlorine nonionic; negative ferric chloride, Sakaguchi, Pauly, Molisch, biuret, Benedict [2, 10]; not hydrolyzed by papain, trypsin, chymotrypsin, pepsin; hydrolyzed by bacterial enzymes from old cultures of *P. vulgaris*, *B. subtilis*, *B. mycoides* and *E. coli*.[14]

Assay. Turbidimetric with *S. paradysenteriae* [1, 10, 11, 18]; with *Sh. sonnei* [15]; paper disc-plate with *B. subtilis* [2, 15]; serial dilution with *Sh. sonnei* [15]; colorimetric [16]; cylinder plate with *Sar. lutea*.[17] Turbidimetrically, 50% inhibition of *S. pardysenteriae* by 0.2 γ/ml [10]; by paper disc-plate, 630 streptomycin units/mg with *B. subtilis*.[2]

Spectrum in vitro. Inhibition concentration (γ/ml) [9, 19, 20, 21, 23]:

Aero. aerogenes	0.5–2.5	*G. tetragena*	2.5
Alc. fecalis	1.0	*H. pertussis*	0.2–0.3
Alc. metalcaligenes	200–>200	*K. pneumoniae*	0.5–15.0
B. anthracis	0.75–5	*Malleo. mallei*	37.5
B. mycoides	2.5	*Staph. citreus*	2.5–5
B. subtilis	10	*Staph. albus*	1.0–5
Br. abortus	2.0–10	*Staph. aureus*	5–10
Br. bronchisepticus	10	*Myco. phlei*	25
Br. melitensis	0.5–5	*Myco. tuberculosis*	6.25–25
Br. suis	0.5–5	*N. catarrhalis*	0.5–2.5
Bor. recurrentis	2.5	*N. meningitidis*	2.5
Clostridia	>500	*Past. avicida*	0.25–0.5
C. diphtheriae	0.5	*Past. bollingeri*	0.25–0.75
C. diphtheroides	1.0	*Past. bubalseptica*	0.5
C. pyogenes	2.5	*Past. cuniculicida*	0.5
D. pneumoniae	1.0–2.5	*Past. oviseptica*	2.5
E. coli	2.5	*Past. tularensis*	0.4–10
(streptomycin resist.)	0.5	*Pr. vulgaris*	1.0–5
Ps. aeruginosa	10–>200	*Proteus* sp.	5–25
S. enteriditis	0.75–2.5	*Sar. lutea*	0.5–10
S. gallinarum	2.5	*Sar. aurantica*	1.0
S. paratyphi	0.75	*Scr. marcescens*	2.5–5
S. schottmulleri	0.5–2.5	*Sh. dysenteriae*	0.75
S. typhimurium	2.5–5	*Sh. madampensis*	0.5–2.5
S. typhosa	0.75–5	*Sh. sonnei*	2.5–5
St. hemolyticus	0.75–2.5	*St. lactis*	0.75
St. infrequens	2.5	*St. non-hemolyticus*	0.25–0.75

V. comma 1.0

No activity against yeasts, fungi, protozoa,[9, 19] *Histoplasma capsulatum.*[24] Immobilization of *Borrelia novyi* and *recurrentis* at 2.5–50 γ/ml; possible inhibition of *Endameba histolytica;* other species not inhibited.[19]

Factors affecting activity. Chloramphenicol is bound 45% by serum albumin.[9]

Spectrum in vivo. *Experimental infections in animals.* In mice, chick embryos and guinea pigs, favorable results were obtained in experimental infections with lymphogranuloma, psittacosis, typhus, murine typhus, scrub typhus, rickettsial pox, Rocky Mountain spotted fever, Q fever.[9, 19, 25, 26] In mice, 35–50 mg/kg protected 50% against *K. pneumoniae;* over 80 mg/kg was needed for protection against hemolytic streptococcus A [27]; 7.6 mg intravenous or 14.9 mg oral gave 50% protection against *Borrelia novyi* [19]; 2.5 mg subcutaneously up to 6 hours after infection gave complete protection against *V. comma.*[28] In embryonic eggs, 1.0 mg, and in mice 1.5 mg intraperitoneal or oral protected against *Rickettsia orientalis*, *R. akari*, *R. prowazeki*, *R. mooseri*, *Dermacentroxenus rickettsi* or psittacosis virus.[29]

In mice, moderate protection against *Klebsiella* and *Shigella*.[9] In mice, chick embryos, guinea pigs and other animals, chloramphenicol was ineffective against tuberculosis,[23] avian malaria (in ducks), *T. pallidum*, pneumococcus, streptococcus, *Plasmodium lophurae*, *Endameba histolytica*, influenza A, St. Louis encephalitis, rabies, vaccinia, variola, Japanese encephalitis, poliomyelitis, intestinal virus, mumps, distemper, Newcastle disease.[9, 19, 29]

Clinical. Effective against Rocky Mountain spotted fever at 50–128 mg/kg followed by 0.25–0.5 g every 3 hours, orally [30]; favorable results in typhus, 40 mg/kg followed by 35 mg/kg daily, orally [31]; in typhoid fever, 50–100 mg/kg followed by 100–250 mg/kg daily, orally [32, 33]; and in scrub typhus, 50 mg/kg followed by 0.2–0.3 g/kg every 2–4 hours.[39] Beneficial effect in brucellosis,[33, 34] bacterial pneumonias,[35] salmonellosis and carriers in children,[36] acute gonorrheal arthritis in males,[37] and in mixed bacterial infection of urinary tract in conjunction with sulfadiazine or penicillin.[38]

PHARMACOLOGY

Toxicity. LD 50 (mg/kg) [9, 13]

Mice:	intravenous	109.5–202.6, 245
	intraperitoneal	1,320
	oral	2,640
	subcutaneous	100 daily, tolerated
Rats:	intravenous	175.5–278.0
Rabbits:	intravenous	117
	subcutaneous	100 daily, tolerated

	MLD	(mg/kg)
Dogs:	intravenous	150
	intramuscular	>46.5–101
	oral	>300

	MLD	(mg/kg/day)
Mice:	oral	385
	preoral	425
	subcutaneous	100
	intraperitoneal	250
Guinea pig:	oral	250
Dogs:	oral	>200–300
	intramuscular	>74–93
Rabbits:	intramuscular	>100

Clinically, no toxic symptoms with 2.0 g orally or 1.0 g per day for 10 days.[22]

Blood levels, Excretion. In mice, 100 mg/kg subcutaneously twice daily, produced 11 γ/ml in the blood and 60 γ/ml in the urine. In guinea pigs, 750 mg preorally, yielded 2.3–7.8 γ/ml in the blood at 2 hours. In dogs, 2 hours after administration [9, 13]:

	mg/kg	Serum γ/ml	Urine γ/ml
intravenous	19–50	7	644
intramuscular	90–101	7	114
oral	86–150	8	283

Concentration in urine maximal at 1,500 γ/ml, 2–8 hours after 86–150 mg/kg administered orally; only 3.5–8.7% of chlorampenicol accounted for in urine [13]; renal clearance not influenced by caronamide.[40]

Clinically, 2.0 g orally, produced after 2 hours, 10 γ/ml in the blood and 380–670 γ/ml in the urine [22]; blood levels of 20–80 γ/ml obtained by 50 mg/kg initially and 0.2–0.3 g orally every 2–4 hours.[32, 39]

Bile levels. After 75 mg/kg, levels of 10–30 γ/ml were obtained.[13]

Absorption, Diffusion. Rapidly absorbed from oral administration [16]; diffused through placenta and into spinal fluid; absorbed into circulation from rectal administration in infants.[40]

Physiology. In dogs, intravenously, 12.5–25 mg/kg had no effect on blood pressure; 100 mg/kg caused a fall in blood pressure, decreased amplitude and increased rate of respiration.[13]

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CHLORELLIN

First described by Pratt and Fong in 1940[1,2]; derived from *Chlorella vulgaris* and *Ch. pyranoidosa.*

PRODUCTION

Fermentation. Photosynthesis on medium containing inorganic salts and carbon dioxide under visible light.[1,2,3,4]

Isolation. Culture liquor extracted with chloroform, dichloroethane or benzene; solvent evaporated and taken up in water.[4]

BACTERIOLOGY

Inhibition of growth of *Ch. vulgaris* (chlorellin-producer) [2]; of *Staph. aureus, St. pyogenes, B. subtilis, E. coli* and *Ps. aeruginosa.*[4]

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"CHLOROMYCETIN" See CHLORAMPHENICOL

CHLORORAPHIN

Antibacterial activity first described by McIlwain in 1941; derived from *Chromobacterium* sp. and by synthesis from nitrobenzene and anthranilic acid.[2]

CHEMISTRY

Constitution. $C_{28}H_{20}O_2N_6$; a quinhydrone-like compound of phenazine-α-carboxylic amide and its dihydro-compound.[1, 2]

Properties. Green pigment; m.p. 225–230° C (dec.).[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (γ/ml):

Staph. aureus	>100	*Eb. typhi*	>100
St. hemolyticus	100	*Pr. vulgaris*	>100
E. coli	>100		

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CIRCULIN

First described by Murray and Tetrault in 1948 [1]; derived from *Bacillus circulans*. The name CIRCULIN was coincidentally used to describe the antibiotic now designated POLYPEPTIN.[3]

PRODUCTION

Fermentation. Submerged growth in shaker flasks and deep tanks on medium containing dried brewer's yeast 2%, dextrin 2%, ammonium sulfate 1%, $CaCO_3$ 1.6%, KCl 0.4%, KH_2PO_4 0.02%; incubated 3 days at 24° C, final pH 7.8; yield in shaker flasks 2,160 *S. typhosa* units/ml. In 100-gallon tank at 270 rpm and 0.6 volume air per minute/volume medium ratio; yield 1,260 *S. typhosa* units/ml.[2, 7]

Isolation. Culture filtrate adjusted to pH 6.5 with sulfuric acid, adsorbed on activated carbon, the carbon cake washed with water and with 50% aqueous tertiary butanol; activity eluted with 25% aqueous tertiary butanol maintained at pH 2.5–4.0 with sulfuric acid; eluates concentrated in vacuo, neutralized with barium hydroxide, filtered and the filtrate freeze-dried; yield, a cream-colored product assaying 800 units/mg representing 30% recovery.[2]

Purification. By chromatography of circulin sulfate over activated carbon using 25% aqueous tertiary butanol as the developer; yield, 47% recovery from crude circulin or 15% from culture liquor with potency up to 2,700 units/mg; highest potency obtained, up to 5,000–5,500 units/mg, by repeated carbon chromatography [2]; maximum potency 6,000 units/mg.[4]

CHEMISTRY

Constitution. A basic peptide, containing *d*-leucine, *l*-threonine, *l*-α,γ-diaminobutyric acid and an optically active fatty acid isomeric with pelargonic.[6]

Properties. Soluble in water and lower alcohols; insoluble in hydrocarbons, chlorinated hydrocarbons and ethers; optical activity: levorotatory; dialyzes readily through cellophane membrane.[2]

Reactions. Positive biuret, Van Slyke, formalin, 2,4-dinitrofluorobenzene; negative ninhydrin; stable at pH 2.5–6.5; thermostable; unstable above pH 7.0 in aqueous solution; inactivated by crude trypsin but not by pepsin; forms HCl, reineckate and helianthate salts.[2,6]

BACTERIOLOGY

Assay. Serial dilution with *S. typhosa;* plate method using filter paper discs with *E. coli.* Samples prepared by boiling for 10 minutes and diluting with pH 2.0 glycine-HCl buffer.[2]

Spectrum in vitro. Inhibition concentration (γ/ml)[2]:

Aero. aerogenes	3.1	*Neis. catarrhalis*	3.1
B. anthracis	100	*Prot. vulgaris*	67.0
B. subtilis	>100	*Pseudo. aeruginosa*	3.1
Brucella abortus	0.3	*Salmonella* sp. group B	1.6
Brucella bronchiseptica	0.8	*S. enteritidis* (several strains)	1.6
E. coli	0.8	*S. gallinarum*	0.4
Kleb. pneumoniae	3.1	*S. paratyphi* (several strains)	6.2
Micro. pyogenes var. *aureus*	100	*S. pullorum*	0.8
Myco. avium	100	*S. schottmuelleri*	6.2
Myco. tuberc. hominis	30	*S. typhimuium*	1.6
S. typhosa (several strains)	3.1	*Shigella dysenteriae*	<0.4
Serratia marcescens	30.0	*Strept. fecalis*	10.0

Spectrum in vivo. In mice experimentally infected with *S. typhosa,* 10 mg/kg subcutaneously gave 100% protection; with *K. pneumoniae,* 16 mg/kg intraperitoneally gave practically 100% protection [2,7]; with *V. cholera* by one-seventh to one-fourth of LD 50 dose.[7]

PHARMACOLOGY

Toxicity. LD 50 mg/kg [2, 5]:

	6,000 μ/mg	2,700 μ/mg
Mice: intravenous	10	23
subcutaneous	77	150–180
intraperitoneal	—	68
Rats: intravenous	—	20
intramuscular	23	70
oral	>200	—

No evidence of chronic toxicity by repeated doses in mice and guinea pigs[2, 5]; mice receiving a total dose of 33 mg/kg subcutaneously over 14 days showed 80% survival; skin sloughing at site of injection; no specific kidney toxicity.[5]

Blood levels. In dogs, administration of 2.5–5 mg/kg intravenously and intramuscularly appeared in the blood at 70 units/ml shortly after injection, falling to 5–20 units/ml at 6 hours. Orally administered, 40 mg/kg resulted in blood levels of 30–60 units /ml at 1–6 hours. No blood levels were obtained in rats following oral administration of up to 200 mg/kg.[5]

Excretion. From 6–10% of circulin administered orally or parenterally appeared in the urine of dogs.[5]

Physiology. Circulin intravenously lowered blood pressure in cats and dogs; no effect on heart or respiratory rate.[5]

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CITRININ

First described and prepared in pure form by Hetherington and Raistrick from *Penicillium citrinum* in 1931[1]; also derived from *P. expansum*, *P. lividum*, *P. phoeo-janthinellum*, *P. implicatum*, *P. chrzaszszi*, *P. citreo-sulfur-*

atum[26]; *Aspergillus terreus*,[4] *Aspergillus* sp. of *Candidus* group[11,16]; found in the leaves of *Crotolaria crispata*, a flowering plant of Northern Australia.[3] NOTALIN is a therapeutic agent derived from fermentation liquor whose major component is citrinin.[19]

PRODUCTION

Fermentation. *P. citrinum.* Surface growth (A) on Czapek-Dox glucose medium incubated at 28° C 12–22 days, yield 1.5–2 g/l[1]; (B) on medium g/l glucose 50, $NaNO_3$ 2, KH_2PO_4 1, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; incubated 14 days at 24° C, yield 2.2 g/l[5,8]; (C) on asparagus butt juice medium with 10 days' incubation at 30° C; yields in excess of 2 g/l[13,20,31]; also on media containing sugars, milk, gelatin, bread, potato.

A. terreus. Surface growth on Czapek-Dox medium with 5% glucose incubated 35 days at 24° C.[4]

Aspergillus sp. of *Candidus* group, surface growth (A) on medium g/l carbohydrate 40, $NaNO_3$, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.005, $ZnSO_4 \cdot H_2O$ 0.005, pH 3.9–4.2; incubated 20 days at 26° C; yields g/l on glucose 2.185, on levulose 2.890, on sucrose 2.755, on maltose 1.955, on honey 4.255, on 8% glucose incubated 20 days at 28° C 3.860; incubated 28 days 4.189, on honey with 28 days' incubation 4.250; decreased yields at 37° C and 40° C[11]; (B) on Czapek-Dox medium containing maple syrup 100 g/l at 37° C, yield 4.5 g/l in 10 days, 5.5 g/l in 15 days; additional yields by reflooding drained mycelium with fresh medium.[16]

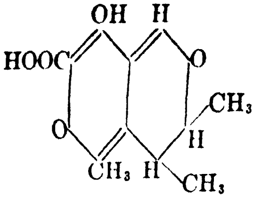
Isolation. Filtered culture medium and washings from mycelium acidified to pH 2.0–3.0 with concentrated HCl; crude citrinin crystals separated, washed with water, dried.[1,4,5,8,20]

Crotolaria crispata dried leaf powder. (A) Extracted with dilute alkali, filtrate acidified to precipitate citrinin; yield 2 per cent. (B) Digested repeatedly with dilute sodium carbonate, the extract expressed and acidified, precipitating 1.4 per cent of nearly pure citrinin. (C) Extracted with warm ether, the extract evaporated to dryness; the residue dissolved in hot alcohol, precipitating citrinin on cooling; yield 0.52 per cent. (D) Extracted with ether in Soxhlet, the extract evaporated to dryness; the residue digested with dilute sodium carbonate and the solution filtered; the filtrate acidified, the precipitate extracted with dilute sodium carbonate and reprecipitated with acid; yield 1.06–1.21 per cent.[3]

Purification. By recrystallization from boiling absolute alcohol, yield 40–45%[1] increased to 60–70% when 3–4 times its volume of water is added to alcoholic solution[11]; by recrystallization from dioxan, yield 70–80%[8,11]; by sublimation[18,20]; by recrystallization from hot ethylacetate followed by aldehyde-free ethanol, yield 75%[20]; by extraction with ether and evaporation of extract to dryness.[4]

CHEMISTRY

Constitution. $C_{13}H_{14}O_5$; proposed structure [2, 18, 22, 28, 32, 33]:



Confirmed by synthesis [28, 33]

Molecular weight by analysis 250, equivalent weight by titration 245 in water and 254 in alcohol; 259 by b.p. depression in dioxan.[4]

Properties. Crude citrinin appears as a yellow green powder [4]; golden-yellow or golden-buff prismatic needles or serrate plates when recrystallized from alcohol, citron-yellow crystals from dioxan,[11] bright lemon color by sublimation,[13] green-yellow fluorescence under ultraviolet [20]; m.p. crude 168° C (decomp.) [5]; m.p. from alcohol crystallization 166–170° C,[1] 173° C with decomposition,[4] 170–171° C,[7] 168–170° C,[11] 171–172° C (uncorr.) [26]; crystallized from dioxan m.p. 170° C.[11]

Solubility % at 25° C:

petroleum ether (b.p. 35–60° C)	0.056
ethyl ether	0.578
ethyl alcohol 95%	0.708
ethyl alcohol 95% (hot)	5
ethylacetate (b.p. 77° C)	2.11
ethylacetate (b.p. 77° C — hot)	20
benzene (b.p. 80° C)	2.858
acetone	4.94
chloroform	7.74
water	insoluble

Soluble in aqueous NaOH or carbonate with evolution of CO_2 and formation of orange-yellow solution; soluble with decomposition in aqueous sodium acetate [1]; $[\alpha]^{23°}_{5461} = -43.1$ (c = 0.92 in absolute alcohol) [4]; density of solution with ferric chloride under standard conditions, proportional to citrinin content over range 0–600 mg/l [25]; polarographic analyses.[7, 10]

Reactions. In aqueous NaOH orange-yellow solution [1, 8] changing to orange-red on standing [1]; buff-colored precipitate solution from solution of sodium salt with ferric chloride, soluble in excess of reagent giving characteristic deep iodine-brown solution [4]; aqueous solution below pH 4.6 bright lemon-yellow (green fluorescence), fading at 4.6, changing to orange pink at pH 5.6–5.8 and to cherry-red above pH 9.9 [7]; solution in 95% alcohol with 3% H_2O_2, intense yellow solution, changing to colorless to light brown, then to deep wine-red with 0.2*N* NaOH; same reaction given by citrinin in culture medium as well as by acid-precipitation [8]; positive reaction to Tauber's color test [8] and to ferric chloride test [11]; couples readily with diazotized chloro-aniline, 2,5-dichloraniline, sulfanilamide.[19]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* citrinin dissolved in sodium citrate 1.05%, NaCl 0.8% or in potassium phosphate buffer at pH 7.0 or in water adjusted to pH 8–10 [12]; activity in serial dilution unaffected by autoclaving [29]; cylinder plate assay with *Staph. aureus* or *E. coli.*[21]

Spectrum in vitro.

	Inhibition dilution [5, 12, 16, 29,]
Staph. aureus (several strains)	10,500–160,000
Staph. albus (2 strains)	30,000– 50,000
Staph. citreus (2 strains)	24,000– 80,000
Strep. hemolyticus A (type 1, 2, 3)	12,500– 18,500
Strep. viridans	9,000 (Na salt)
B. subtilis (several strains)	30,000–200,000
B. mycoides	128,000
E. coli	2,000
E. coli commune	24,000, 9,000 (Na salt)
Eb. typhi	2,000, 9,000 (Na salt)
Eb. typhimurium	4,000, 9,000 (Na salt)
Shig. paradysenteriae	9,000 (Na salt)
Shig. dysenteriae	24,000
Sal. enteriditis	24,000
Sal. paratyphi	9,000 (Na salt)
Proteus vulgaris	24,000, 9,000 (Na salt)
Ps. aeruginosa	4,000–8,000
V. cholera	50,000

	Inhibition γ/ml[23]
Staph. aureus	16
E. coli	71,000
B. subtilis	16
B. mycoides	32
Photo. fischeri	16
Myco. phlei	125
Myco. smegmatis	250

Spectrum in vivo. No protection for mice against lethal doses of *Staph. aureus* at one-half of toxic dose.[19]

Factors affecting activity. Antibacterial action is bacteriostatic and bactericidal,[12] is enhanced by 1% glucose (due to acid production), reduced by fresh horse serum 5%, unaffected by sucrose 1% and *p*-aminobenzoic acid 20 mg/ml.[12]

PHARMACOLOGY

Toxicity [12, 20]

Mice:	subcutaneous and intraperitoneal	LD 50 = 35 mg
	intraperitoneal	MLD = 2 mg/kg
	intraperitoneal	100% mortality at 400 mg/kg
	subcutaneous	LD 80 = 2 mg
	oral	no effect at 100 mg/kg
Rats:	subcutaneous and intraperitoneal	LD 50 = 67 mg
	subcutaneous and intraperitoneal	MLD = 50 mg/kg
	subcutaneous	100% mortality at 100 mg/kg
	intraperitoneal	100% mortality at 75 mg/kg
Guinea pigs:	subcutaneous and intraperitoneal	LD 50 = 37 mg
	subcutaneous	100% mortality at 75 mg/kg
Rabbits:	oral	no effect at 25.7–90 mg/kg
	subcutaneous	no effect for 5 days at 30 mg/kg/day
	intravenous	LD 50 = 19 mg

On autopsy, kidney pathology observed particularly in the proximal convoluted tubule; no subacute toxicity in rabbits; no effect on red and white cell counts; no hemolysis; no agglutination [20]; on human and rabbit skin, no local irritation by topical administration of pure powder, 1–5% ointments, 1–20% aqueous solutions of the sodium salt [19, 20]; intradermally in human and rabbit, slight to no effect by 1–2% of aqueous sodium salt; some irritation of mucous membranes by 0.1–0.2%.[19]

Absorption. From mucous membranes, negligible; definite absorption from alimentary canal giving citrinin levels in blood and urine.[19]

Physiology. Ciliary activity of frog esophagus depressed by 0.6–0.8% of sodium salt; little or no effect on sensory and motor nerve irritability at 0.1–0.8% [20]; on tissues and organs isolated and in situ, citrinin acts like a parasympathetic stimulant similar to acetylcholine and pilocarpine.[19, 20]

MISCELLANEOUS

Citrinin solutions, adjusted to approximately pH 7, were toxic to amoebae, flagellates and ciliates at a dilution of 1:1,500 [17] and to paramecium at concentration greater than 1:5,000.[19]

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CLAVACIN

First described and named by Waksman, Horning and Spencer in 1942[1]; simultaneously observed by Wiesner[2] and by Wilkins and Harris[3]; described and named CLAVIFORMIN by Chain, Florey and Jennings.[4] Also described as PATULIN, EXPANSIN,[5] CLAVATIN[6]; identified with each other and with clavacin.[7, 8, 9, 10] Derived from *Aspergillus clavatus*,[1, 2, 6, 10, 11, 12] *A. terreus*,[13] *A. giganteus*,[18] *Penicillium claviforme*,[4, 6] *P. patulum*,[5, 14] *P. expansum*,[5, 13] *P. divergens*,[15] *P. urticae*,[13] *P. leucopus*,[12] *Penicillium* sp.[12, 16, 17] and *Gymnoascus* sp.[16]

PRODUCTION

Fermentation. Surface growth with *A. clavatus* on medium containing g/l (A) glucose 40, $NaNO_3$ 3, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; incubated at 28° C for 6–7 days, final pH 4.5; yield 250 *S. lutea*, 100 *E. coli*, 80 *B. mycoides* units/ml[11]; (B) glucose 40, $NaNO_3$ 3, $KHPO_4$ 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.01, yeast extract 10 or preferably corn steep liquor 2 ml; incubated at 23–25° C for 5–11 days.[10] With *A. clavatus* on Czapek-Dox.[12]

With *A. terreus*, on Czapek-Dox or on 2% malt extract medium.[13]

With *P. divergens*, on modified Raulin-Thom medium.[15]

With *P. urticae*, on Czapek-Dox medium incubated at 24° C for 6–10 days.[13]

With *P. expansum* on potato-dextrose.[13]

With *P. patulum* on Raulin-Thom with 5% glucose.[14]

With *Gymnoascus* sp. on Czapek-Dox with corn steep liquor 3%.[16]

With *Penicillium* sp. on Czapek-Dox[16] with soil extract 0.01 g/l, pH 4.0; incubated at 25° C for 8 days; yield 70–135 *E. coli* units/ml.[17]

Submerged growth with *Penicillium* sp. on Czapek-Dox with soil extract 0.01 g/l, incubated for 8 days; yield 26 *E. coli* units.[17]

Isolation. (A) Activity of culture filtrate extracted with ether or chloroform or adsorbed on activated carbon and eluted with ether or chloroform.[1, 11] (B) Culture filtrate at pH 5 concentrated in vacuo to small volume, extracted with ethyl acetate, extract evaporated to dryness, residue extracted with chloroform and evaporated to dryness.[4, 14] (C) Activity of culture filtrate adsorbed on activated carbon at pH 5.5–6.0; carbon cake dried and extracted in Soxhlet with acetone or preferably chloroform; extract evaporated in vacuo to an oily residue.[10] (D) Culture filtrate concentrated in vacuo, extracted with ether; ether extract dried over sodium sulfate and evaporated to dryness.[12] (E) Activity of culture filtrate adsorbed on activated carbon, eluted with ether-acetone,[15] eluted with 80% acetone, eluate evaporated until acetone-free [13, 16]; eluted with ethyl acetate, eluate evaporated to a gummy residue.[17]

Purification. (A) Crude clavacin dissolved in hot chloroform, cooled and allowed to crystallize; crystals washed with cold ether, recrystallized from chloroform or from benzene-methanol.[4] (B) Dissolved in warm ethanol, cooled acetone added precipitating inert material; filtrate concentrated, additional inert material precipitated by addition of ether; final solution evaporated in vacuo, residue dissolved in warm dry chloroform; chloroform solution fractionated on "Permutit" column; chloroform eluates concentrated and cooled, precipitating crystalline clavacin; recrystallized from chloroform, ether or benzene.[10] (C) Extracted with ether; ether extract concentrated and allowed to crystallize; recrystallized from ether and benzene.[12] (D) Dissolved in ether and passed through an alumina column, yield 25 mg/l.[15] (E) Dissolved in ether, concentrated, cooled and allowed to crystallize; recrystallized from hot chloroform.[13] (F) Crystallized from acetone.[16] (G) Dissolved in warm ethyl acetate, cooled and allowed to crystallize; yield 1.0 g/l.[17] (H) Dissolved in ether, adsorbed on aluminum oxide, eluted with ether, crystallized.[14]

CHEMISTRY

Constitution. Anhydro-3-hydroxymethylene-tetrahydro-1:4-pyrone-2-carboxylic acid; $C_7H_6O_4$; M.W. 154.[5]

Properties. Colorless rhomboid plates,[4] colorless prisms,[10] colorless plates[12]; m.p. 110° C (recryst. from chloroform),[4] 109–110° C,[10] 109.5–110.5° C,[12] 111–112° C,[14] 112° C[17]; sublimed in high vacuum at 80–100° C,[4] at 70° C[10]; soluble in water, alcohol, acetone, chloroform, dilute alkali; less soluble in ether and benzene; insoluble in petroleum ether.[1, 4, 10, 11] Optically inactive[4]; ultraviolet absorption maximum at 2,760 Å,[10] 2,765 Å.[7] $E^{1\%}_{1\,\mathrm{cm}} = 900\text{–}950$.[10]

Reactions. Thermostable; stable in dilute acid, labile in alkali; red color with alkali; yellow color with dilute ammonia, piperidine, asparagine, amino acids and peptones without loss of antibiotic activity; loss of activity by repeated recrystallization; reduces ammoniacal silver nitrate, Fehling, potassium permanganate; yellow crystals with 2,4-dinitrophenylhydrazine.[4, 10, 11, 17]

BACTERIOLOGY

Assay. Serial dilution with *E. coli* [10]; streak plate dilution with *S. lutea, E. coli* or *B. mycoides* [11]; cylinder plate with *E. coli.*[17] Extract with ethyl acetate gave red color with alcoholic ammonia and ethyl cyano-acetate measured at 5,300 Å.[23] Crystalline clavacin 200,000–240,000 *E. coli* dilution units [10]; 48 cylinder plate *E. coli* units per mg.[17]

Spectrum in vitro. Inhibition dilution (× 1000):

Staph. aureus	60–200	*B. mycoides*	200
S. lutea	500	*S. typhi*	160
B. subtilis	200	*S. gaertneri*	80
E. coli	80–300	*Ps. aeruginosa*	<10–40
Aero. aerogenes	50	*St. pyogenes*	40
S. schottmuelleri	60	*S. typhi-murium*	200–240
S. cholerae-suis	150	*S. paratyphi*	200–300
Salmonella sp.	75	*Shig. alkalescens*	120–200
B. megatherium	100	*Pr. vulgaris*	200–300
B. cereus	125	*E. typhi*	400 [4, 11, 12, 17]

T. gypseum	1.0
T. purpureum	1.0
Microsporon audouini	100
Monilia albicans	<0.1–0.1 [20, 21, 22]

Mode of action. Bacteriostatic and bactericidal.[1, 4]

Factors affecting activity. Partial inactivation by 10% serum [4]; no effect on activity by serum.[10]

Spectrum in vivo. In mice, clavacin gave no protection against experimental infection with *S. schottmuelleri* at highest tolerated dose [16]; incubated with tetanus toxin, prior to administration, protected against tetanus.[24, 25, 26, 27]

Clavacin caused temporary inhibition of coagulation of rabbit blood plasma by staphylococci; no effect on coagulation by staphylococcal toxin, on the action of antitoxin against staphylococcus hemotoxin.[28]

Clavacin has been tested clinically as a therapeutic agent for the common cold.[29]

PHARMACOLOGY

Toxicity. In mice [4, 10, 16, 17, 23]:

	Intraven.	Intraperit.	Subcut.	Oral
LD100 mg/20 g	0.25–0.5	0.1–0.2	0.2–2	2.5
LD50 mg/20 g	0.5	——	0.2–0.3	0.7

Subcutaneous and intraperitoneal administration at 0.01 mg per day for 11 days was not lethal; subcutaneous edema followed administration by that route.[23] In rats, the LD50 mg/100 g subcutaneously was 1.5–5.0[10, 23]; with intracutaneous administration, edema and necrosis in rats, rabbits and guinea pigs; inflammation by installation in conjunctival sac in rabbit; with clavacin ointment on artificial wounds in guinea pigs, ulceration and necrosis.[23]

Clavacin acetate in benne oil about one-half as toxic.[10]

Toxic to leucocytes at 1:800,000[4]; inhibition of growth in tissue culture of chick embryo fibroblasts at 1:16,000.[23]

Physiology. In cats, clavacin caused rise in blood pressure followed by considerable fall[4, 23]; in anaesthetized rabbits and dogs, fall of blood pressure, heart acceleration, vasodilatation, contraction of spleen; stimulation of respiratory center; depression of smooth muscle tonus; fall in alkali reserve of plasma; increase in hemoglobin; rise in blood sugar. Clavacin causes a pressor action by stimulation of the vasomotor center of the medulla with peripheral vasodilatation and little direct effect on the heart.[23]

Clavacin, at 0.25 mg/100 g in rats, abolished diuresis, marked antidiuretic effect at lesser dose[10, 23]; at 1:10,000, caused arrest of ciliary movement of frog esophagus and cat trachea.[23]

MISCELLANEOUS

Clavacin was lethal to paramecium at 1:1,000,000; and to "guppies" at 1:100,000.[10]

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CLAVATIN *See* CLAVACIN

CLAVIFORMIN *See* CLAVACIN

CLITOCYBIN

First described by Hollande in 1945 [1]; derived from *Clitocybe gigantea* var. *candida* [2]; composed of clitocybin A and B.[3]

PRODUCTION

Fermentation on yeast extract nutrient medium; mycelium extracted with organic solvents and with water; water-soluble fraction fractionally precipitated with ammonium sulfate; ether-soluble fraction chromatographed on an alumina column.[4]

CHEMISTRY

Properties. Rhombic crystals; m.p. 77° C; soluble in water, ether, ethanol, acetone, chloroform and amyl acetate; unstable at 70–80° C and at pH 7; diffuses readily.[1, 2, 3]

BACTERIOLOGY

Assay. Cylinder plate with *Staph. aureus.*[5]

Spectrum in vitro. Inhibition dilution (× 100,000): [5]

Eb. typhi	3	*B. anthracis*	3
S. paratyphi	3	*St. lactis*	6
E. coli	3	*St. pyogenes*	6
Br. abortus	4	*Myco tuberculosis*	8

At 1:400,000–800,000, active against *Staph. aureus*, *B. anthracis*, *Erysipelothrix rhusiopathiae*, *S. schottmuelleri*, *Past. avicida*, *Malleomyces mallei* and *Myco. tuberculosis bovis* [4]; active against *Cl. perfringens*, *Ps. aeruginosa* and some infusorial protozoa.[1, 5]

Spectrum in vivo. In guinea pigs, some protection was provided against experimental infections with tuberculosis, staphylococcus, streptococcus, *Pasteurella* and the virus of foot-and-mouth disease.[1–6]

PHARMACOLOGY

Toxicity. In guinea pig, 20 mg tolerated [3, 4]; toxicity related to activity of clitocybin preparations [4]; impure preparation showed acute and chronic toxicity,[2] edema, hemorrhage and ulceration on subcutaneous administration.[1]

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(5) Rivière, C., Salomon, L., Thély, M., and Gautron, G., "Etude des propriétés bacteriostatiques de la clitocybine," *Ann. Inst. Pasteur*, **74**, 118 (1948).

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COLICINE

First isolated by Heatley and Florey in 1946 [1] from an *E. coli* strain isolated from cat feces; named from the antibiotic complex produced by various strains of *E. coli* and designated "colicines" by Gratia and Fredericq in 1946.[2]

The antibacterial activity of *E. coli* has been noted by many observers prior to 1946.[1]

PRODUCTION

Fermentation. Submerged fermentation by bubbling air through the culture liquor, on medium containing g/l "Lab. Lemco" 10, peptone 10, pH 6.8; lard or arachis oil antifoam; incubated for 16 hours at 37° C; final pH 8.5.[1]

Isolation. Activity of the culture filtrate adsorbed on activated carbon, the carbon washed with water, with boiling 80 per cent alcohol, and dried; activity eluted with ether, glacial acetic acid, phenol or possibly pyridine; eluates evaporated in vacuo to a syrup, the syrup ground with sand and precipitated with excess absolute alcohol; the precipitate washed with alcohol and dried at 37° C; yield 0.6 g/l culture liquor.[1]

Purification. Crude colicine extracted with water at pH 8.0–8.5, precipitated with phenol; the phenol precipitate stirred with water at pH 8.0 and washed with ether; the aqueous layer dried in a desiccator to a brown residue; yield 10–20 mg/l culture liquor.[1]

CHEMISTRY

Constitution. Probably a peptide structure.[1]

Properties. Odorless, faint gelatin-like taste; soluble in water, acetic acid; slightly soluble in pyridine, wet phenol; foams in solution; dialyzable through cellophane; thermostable in acid or neutral solution; less stable in alkali; destroyed by pepsin, trypsin, mouse kidney and liver tissue, staphylococcal pus.[1]

BACTERIOLOGY

Assay. Cylinder plate method with a sensitive strain of *E. coli.*[1]

Spectrum in vitro. Active against *S. typhi, Sh. flexneri, C. xerosis, Myco. phlei, Vibrio comma, Ps. aeruginosa, S. typhimurium, S. newport, Sh. shigae, Sh. sonnei, S. enteritidis, S. paratyphi.*[1,3]

Mode of action. Bactericidal.[1]

Factors affecting activity. Activity decreased with increased inoculum, serum.[1]

PHARMACOLOGY

Toxicity. In mice, intravenous, 18 mg was tolerated; in another batch of colicine, 5 mg was lethal; human leucocytes unaffected by colicine 1:1,000; no hemolysis of human red cells.[1]

Excretion. Little or no activity detected in the urine of mice after intravenous administration.[1]

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(3) Gratia, A., Joiris, E., and Weerts, E., "Distribution of colicine-producing bacteria in human and animal feces," *Rept. Proc. 4th Intern. Congr. Microbiol.*, 141 (1947).

COLISTATIN

First described by Gause in 1946; [1] named from its bacteriostatic action on *E. coli;* derived from an aerobic sporulating bacillus isolated from soil.

PRODUCTION

Fermentation. Surface culture on medium g/l glucose 30, sodium nitrate 3, KH_2PO_4 1.5, $MgSO_4$ 0.5, $FeSO_4$ 0.015, "Tryptone" 1.0, pH 6.5; 3 days' incubation at 28° C, yield 2,500 *Staph. aureus* units/ml.[1]

Isolation. Culture fluid acidified to pH 3.5, treated with activated carbon, filtered, neutralized, active portion of filtrate adsorbed on activated carbon, eluted with 80% aqueous acid methanol or ethanol, eluate neutralized, solvent removed in vacuo at 50° C, aqueous concentrate evaporated to dryness in vacuo, or crude colistatin precipitated with acetone.[1]

CHEMISTRY

Properties. Crude colistatin soluble in acid methanol, less soluble in acid ethanol, insoluble in butanol, precipitated from acid methanol solution by ether, with partial inactivation; colistatin in culture liquor stable to boiling for 15 minutes.[1]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* diffusible through agar.

Spectrum in vitro. Inhibits growth of *Staph. aureus,* pneumococci, *B. proteus, B. paratyphosus* B, *B. dysenteriae* Shiga, and *B. typhi abdominalis.*

Spectrum in vivo. Mice infected with *Borrellia sogdianum* treated with total of 90,000 units/kg show total absence of spirochetes in blood on following day.[1]

PHARMACOLOGY

Concentrated aqueous solution not toxic for mice administered subcutaneously, intramuscularly, intravenously at 100,000 units/kg.[1]

BIBLIOGRAPHY

(1) Gause, G. F., "Colistatin: a new antibiotic substance with chemotherapeutic activity," *Science,* **104,** 289 (1946).

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CORTICIN

First described by Robbins, Kavanagh and Hervey in 1945; derived from a *Basidiomycetes* sp.

(1) Robbins, W. J., Kavanagh, F., and Hervey, A., "Discovery of six new antibiotics," *J. N. Y. Bot. Garden,* **46,** 130 (1945).

CORYLOPHILIN *See* PENATIN

CREPIN

First observed by Osborn in 1943 [1]; isolated and named by Heatley in 1944 [2]; derived from *Crepis taraxacifolia* and probably from *C. virens.*

Crepin occurs in the buds, flowers, inflorescence stems and roots but not in the stem and leaves, as an antibiotically inactive precursor. Conversion to the active form is accomplished by incubation of the aqueous extract with a concomitant enzyme present in the yellow petals and to a lesser extent in the root. Neither precursor nor enzyme is found in the seed.[1]

PRODUCTION

Isolation. Macerated fresh or dried flowers and buds extracted with water, aqueous extract acidified to pH 2.5–3.0 with HCl, resulting precipitate stirred with $N/100$ HCl and centrifuged, activity of the supernatant liquid adsorbed on activated carbon and eluted with 80% acetone; eluate concentrated in vacuo, pH adjusted to 6.0–6.5 and extracted with ether; ether extract passed through an alumina column and refrigerated, precipitating crystalline crepin; yield 100 mg per kg of fresh starting material.[2]

Purification. By recrystallization from absolute and 50% alcohol.[2]

CHEMISTRY

Constitution. Proposed $C_{14}H_{16}O_4 \cdot H_2O$ when recrystallized from 50% alcohol; $C_{15}H_{18}O_4$ from absolute alcohol; M.W. 236–275 (Barger), 266–270 (X-ray).[2, 3]

Properties. Monoclinic, orthorhombic prisms, or orthorhombic prisms terminated by pyramids; odorless, tasteless; darkens at 300° C without melting; slightly soluble in water (0.25 mg/ml); soluble in pyridine, alcohol, ether and glycerol; neutral reaction in water; no characteristic ultraviolet spectrum; stable in acid solution, unstable in alkaline.[2, 3]

Reactions. Negative with ammoniacal silver nitrate, Fehling, 2,4-dinitrophenylhydrazine, ferric chloride, acidified potassium iodide; decolorizes potassium permanganate, bromine water; readily hydrogenated.[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution[2]:

Staph. aureus	32,000	*B. subtilis*	16,000
Str. pyogenes	8,000	*Ps. pyocyanea*	<4,000
S. typhi	4,000		

Factors affecting activity. Activity lowered by human serum.[2]

PHARMACOLOGY

Toxicity. Inhibits human leucocytes at 1:4,500,000; rapidly lethal at 1:4,500.[2]

BIBLIOGRAPHY

(1) Osborn, E. M., "On the occurrence of antibacterial substances in green plants," *Brit. J. Exp. Path.*, **24**, 227 (1943).

(2) Heatley, N. G., "An antibiotic from *Crepis taraxacifolia* (Thuill.)," *Brit. J. Exp. Path.*, **25**, 208 (1944).

(3) Rogers, W., "A note on the X-ray crystallography of crepin," *Brit. J. Exp. Path.*, **25**, 212 (1944).

CURLING FACTOR *See* GRISEOFULVIN

CYNEMATIN

First described by Gottshall, Roberts and Portwood in 1949; derived from a strain of *Tilachlidium.*

BIBLIOGRAPHY

Gottshall, R. Y., Roberts, J. M., and Portwood, L. M., "Cynematin, an antibiotic produced by a strain of *Tilachlidium*," *Soc. Am. Bact.*, **15**, No. 5, 11 (1949).

DICOUMAROL

Antibacterial properties first described by Goth in 1945.[1]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilutions:[2,3]

Staph. aureus	100,000	*B. subtilis*	<25,000
Strep. pyogenes	100,000	*Cl. welchii*	<25,000
Strep. viridans	100,000	*B. abortus*	25,000
Strep. fecalis	<25,000	*Eb. typhi*	<25,000
B. anthracis	100,000–500,000	*E. coli*	<25,000
C. diphtheriae	25,000	*S. paratyphi*	<25,000
Pr. vulgaris	<25,000	*Ps. aeruginosa*	<25,000

Factors affecting activity. Antibiotic activity is lowered by cystein,[3] rutin, quercetin and quercitrin[2]; not affected by methyl-1,4-naphthoquinone.[1]

BIBLIOGRAPHY

(1) Goth, A., "The antibacterial properties of dicoumarol," *Science*, **101, 383** (1945).

(2) Naghski, J., Copley, M. J., and Couch, J. F., "Effect of flavonols on the bacteriostatic action of dicoumarol," *Science*, **105**, 125 (1947).

(3) Brodersen, R., and Kjaer, A., "The antibacterial action and toxicity of some unsaturated lactones," *Acta Pharmacol. Toxicol.*, **2**, 109 (1946).

DIPLOCOCCIN

First observed by Whitehead in 1933[1]; isolated and named by Oxford in 1944[2]; derived from a lactic acid-producing streptococcus.

This antibiotic is unusual in that the organism most susceptible to diplococcin is a streptococcus which is practically indistinguishable from the diplococcin-producing streptococcus.[2,3]

PRODUCTION

Fermentation. Surface growth on culture medium containing g/l $NaNH_4HPO_4$ 49, KH_2PO_4 8.7, $MgSO_4 \cdot 7H_2O$ 5.0, KCl 5.0, $FeSO_4 \cdot 7H_2O$ 0.1, sucrose 30, to which is added 250–500 ml of heart broth, pH 7.8; incubated 11 days at 24° C.[2]

Isolation. Culture liquor supernatant decanted and discarded, cells centrifuged and dried in vacuo; dried bacteria milled, washed by refluxing with water containing a few drops of acetic acid, extracted by refluxing with 0.4% acetic acid.[2]

Purification. Precipitation with picric acid, decomposed with acetic acid in ethanol.[2]

CHEMISTRY

Contains N 13.9%, no S or P; gives all protein tests except Hellers and Millon; soluble in water, insoluble in absolute ethanol; levorotatory; precipitated by half-saturated ammonium sulfate and by tannic acid; negative Molisch; gives tests for arginine, tyrosine, tryptophane; diffuses slowly through collodion membrane; stable in acid, unstable in alkaline solution.[2]

BACTERIOLOGY

Assay. Serial dilution using as test organism a "cheese-starter" streptococcus; purified diplococcin 200 units/mg, picrate 300 units/mg.[2]

Spectrum in vitro. Inhibition dilution [2]:

Strep. cremoris	200,000	*Strep. hemolyticus*	20,000
Strep. lactis	10,000	*Staph. aureus*	10,000
	E. coli	no effect	

BIBLIOGRAPHY

(1) Whitehead, H. R., "A substance inhibiting bacterial growth, produced by certain strains of lactic streptococci," *Biochem. J.*, **27**, 1,793 (1933).

(2) Oxford, A. E., "Diplococcin, an antibacterial protein elaborated by certain milk streptococci," *Biochem. J.*, **38**, 178 (1944).

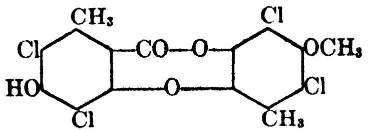
(3) Meanwell, L. J., *Proc. Soc. Agric. Bact.*, **19** (1943).

DIPLOICIN

Antibiotic activity first described by Barry in 1946; derived from the lichen *Buellia canescens*.

CHEMISTRY

Constitution. Proposed structure:



BACTERIOLOGY

Spectrum in vitro. Inhibition dilution:

M. smegmatis	1:70,000
M. tuberculosis	1:100,000
C. diphtheriae mitis	1:100,000

Iodinated and chlorinated phenyl ethers were active in vitro against tuberculosis; a decarboxylated derivative inhibited *C. diphtheriae mitis* at 1:100,000.

BIBLIOGRAPHY

(1) Barry, V. C., "The thyroid and tuberculosis," *Nature*, **158**, 131 (1946).

"DUOMYCIN" *See* AUREOMYCIN

E. COLI FACTOR *See* PENATIN

EI_5

First described by Weiser, Gardner, Lefler, and St. Vincent in 1949; derived from a *Streptomyces* sp.

This antibiotic resembles ACTINORUBIN in antibacterial spectrum, chemical properties and toxicity, although the *Streptomyces* sp. producing EI_5 is distinct from that of actinorubin.

BIBLIOGRAPHY

(1) Weiser, R. S., Gardner, G. M., Lefler, J. S., and St. Vincent, L., "Search for antibiotics active against *Mycobacterium tuberculosis*. 1. A streptothricin-like antibiotic from a *Streptomyces* sp.," *Proc. Soc. Exp. Biol. Med.*, **72**, 283 (1949).

ENDOSUBTILYSIN

First described by Saint-Rat and Olivier in 1946; derived from *Bacillus subtilis*.

PRODUCTION

Isolation. Culture liquor pellicle extracted with alcohol at pH 3; chloroform and half-saturated saline added to alcohol extract; the chloroform layer extracted with dilute NaOH at pH 8–9, the aqueous phase concentrated, adjusted to pH 3 and extracted with chloroform; petroleum ether added to the chloroform extract, the aqueous phase separated and neutralized.

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus*.

Spectrum in vitro. Highly active against *Staph. aureus*, *E. coli*, *Eb. typhi* and *M. tuberculosis*.

PHARMACOLOGY

Low toxicity to rabbit and man.

BIBLIOGRAPHY

(1) Saint-Rat, L., and Olivier, H. R., "Extraction and purification of endosubtilysine," *C. R. Acad. d. sc.*, **222**, 296 (1946).

ENNIATIN A

First described by Gaeumann, Roth, Ettlinger, Plattner and Nager in 1947; derived from *Fusarium orthoceras* var. *enniatinum*,[1] and from *F. sciroi*.[3]

Enniatin A is considered identical with LATERITIIN I.[3]

PRODUCTION

Fermentation. Surface culture with *F. orthoceras* on medium containing g/l glucose 50, ammonium nitrate 10, KH_2PO_4 5, $MgSO_4 \cdot 7H_2O$ 2.5, $FeCl_3 \cdot 6H_2O$ 0.02; incubated 16–18 days at 27–30° C; yield from mycelium, 1,000 *Myco. paratuberculosis* units/ml; only traces of activity in culture filtrate. Submerged culture in shaker flasks, incubated 4–5 days, gave similar yields.[1]

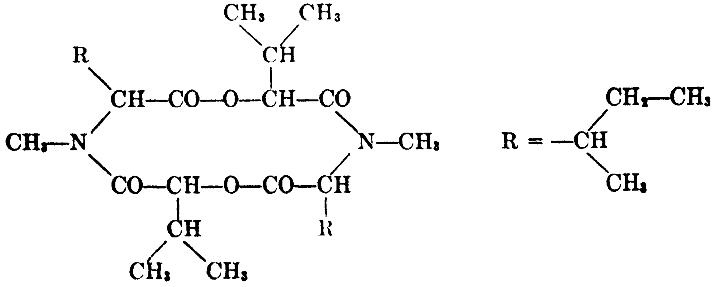
Surface culture with *F. sciroi*, incubated 3–4 weeks at 27° C, final pH 7.5–8.0; yield in culture filtrate 10–14 *Myco. paratuberculosis* units/ml.[3]

Isolation. Mycelium pressed dry, milled with anhydrous sodium sulfate and extracted with ether; ether extract dried and evaporated to a red-brown oil; yield 900 mg/l.[3]

Purification. Activity of ether extract adsorbed on a neutral aluminum oxide column, eluted with benzene-ether, with ether and with ether-acetic ester; eluates diluted with methanol, cooled to minus 15° C, distilled water added precipitating enniatin A; precipitate dried in vacuo over potassium hydroxide and phosphorus pentoxide.[3]

CHEMISTRY

Constitution. Proposed $C_{24}H_{42}O_6N_2$ [1,3,5]:



M.W. 454, 459 (by analysis); 432, 424, 443, 413 (cryo. in benzene), 446 (Barger-Rast).[1,3]

Properties. Long colorless needles; m.p. 122–122.5° C (corr.), 121.5° C (corr.) recrystallized from water-ethanol [1,2,3]; soluble in organic solvents, practically insoluble in water [2]; $[\alpha]_D^{20} = -92°$ (c = *ca.* 1% in chloroform) [1,2]; $[\alpha]_D^{18} = -90°$ (c = 1.0 in chloroform), −91.9° (c = 0.926 in chloroform) [3]; no characteristic ultraviolet absorption spectrum, no polarographic properties; sublimed *in vacuo* without loss of activity.[1,2,3]

Reactions. Thermostable; slowly inactivated by acids, rapidly by alkalies.[1,2,3]

BACTERIOLOGY

Assay. Serial dilution with *M. paratuberculosis*.[1,3]

Spectrum in vitro. Inhibition dilution (× 1,000) [1]:

Myco. paratuberculosis	1,200
Myco. phlei	310
Myco. tuberculosis hum.	100–500
B. subtilis	320
Staph. aureus	160
E. coli	<1

•

BIBLIOGRAPHY

(1) Gaeumann, E., Roth, S., Ettlinger, L., Plattner, P. A., and Nager, V., "Enniatin, a new antibiotic active against mycobacteria," *Experientia*, **9**, 202 (1947).

(2) Plattner, P. A., and Nager, V., "The chemistry of enniatin," *ibid.*, 325 (1947).

(3) Plattner, P. A., Nager, V., and Boller, A., "Wilting agents and antibiotics. 7th communication. The isolation of new antibiotics from Fusaria," *Helv. Chim. Acta*, **31**, 594 (1948).

(4) Cook, A. H., Cox, S. F., Farmer, T. H., and Lacey, M. S., "Production of antibiotics by *Fusaria*," *Nature*, **160**, 31 (1947).

(5) Plattner, P. A., and Nager, V., "Wilting agents and antibiotics. 10th communication. The constitution of enniatin A," *Helv. Chim. Acta*, **31**, 2,192 (1948).

ENNIATIN B

First described by Plattner, Nager and Boller in 1948 [1]; derived from strains of *Fusarium orthoceras* var. *enniatinum*.

PRODUCTION

Fermentation. On culture medium, incubated 14–17 days.[1]

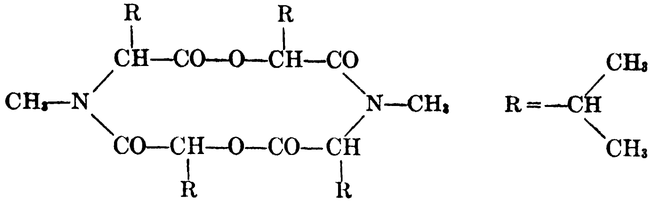
Isolation. Mycelium pressed and dried, extracted with ether; yield of crude enniatin B 1.76% from mycelium, 1.7×10^6 *M. paratuberculosis* units from 5 l of culture liquor (440 units per ml of culture liquor equivalent, at 190 units per mg).[1]

Purification. Chromatography on aluminum oxide yielding crystalline fractions at 190–200 units/mg; yield 0.47–1.7 g/l of culture liquor.[1]

CHEMISTRY

Constitution. Proposed $C_{22}H_{38}O_6N_2$; calculated C 61.94, H 8.98, N 6.57, $2CH_3$ 7.04; found by analysis C 62.35, 61.89, 61.94, H 8.80, 8.69, 8.94, N 6.78, 6.62, 6.66, $2CH_3$ 8.10.[1]

Proposed structure[2]:



No hydroxyl, alkoxyl, carbonyl, or basic or acid functional groups[2]; acid hydrolysis yields 2 moles of N-methyl-(+)-valine and 2 moles of D-alpha-hydroxy-valeric acid; alkaline hydrolysis gives D-alpha-hydroxy-isovaleryl-N-methyl-(+)-valine, the corresponding lactone, and 4-methyl-3,6-diisopropyl-2,5-dioxo-morpholine.[2]

Properties. Of two crystalline fractions from chromatography[1]:

	Recrystallized	m.p. (C°)	α_D Chloroform	units/mg
I	methanol-water	174.5–175.5	−106.3	180
	petrol. ether	175–175.5	−107.9	170
II	petrol. ether	173–173.5	−107.5	130
	ether-pet. ether	173–173.5	−107.1	210

Slightly soluble in water, soluble in most organic solvents.[2]

BACTERIOLOGY

Assay. Serial dilution with *M. paratuberculosis*.[1]

BIBLIOGRAPHY

(1) Plattner, P. A., Nager, V., and Boller, A., "Wilting agents and antibiotics. 7th communication. The isolation of new antibiotics from Fusaria," *Helv. Chim. Acta*, **31**, 594 (1948).

(2) Plattner, P. A., and Nager, V., "Wilting agents and antibiotics. 8th communication. The constitution of enniatin B," *Helv. Chim. Acta*, **31**, 665 (1948).

ENNIATIN C

Enniatin C is considered a mixture of ENNIATIN A and B,[1] or as an isomer of ENNIATIN A.[2]

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(1) Plattner, P. A., Nager, V., and Boller, A., "Wilting agents and antibiotics. 7th communication. The isolation of new antibiotics from *Fusaria*," *Helv. Chim. Acta*, **31**, 594 (1948).

(2) Plattner, P. A., and Nager, A., "Wilting agents and antibiotics. 11th communication. Analysis and characterization of the enniatines. The behavior of N-methyl-amino acids in paper chromatography," *Helv. Chim. Acta,* **31,** 2,203 (1948).

ERYTHRIN

First described by Zil'ber and Yakobson in 1946; derived from animal tissues, apparently from erythrocytes.[1] The name ERYTHRIN has previously been used to describe the erythritol ester of lecanoric acid, derived from the lichen *Roccella tinctoria.*[2]

PRODUCTION

Isolation. Rat liver or spleen extracts by freezing in liquid oxygen with sodium chloride solution; blood of a variety of animals and of man extracted with pH 7.6 phosphate buffer, precipitated with HCl, the precipitate extracted with acetone, the acetone extract concentrated in vacuo and poured into sodium chloride solution to yield erythrin as a dark brown powder.

CHEMISTRY

Properties. Insoluble in water; soluble in ethanol, acetone, sodium carbonate solution, sodium citrate; thermostable.

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (γ/ml):

diphtheria	15–31	Streptococcus	31–62
B. brevis	15–31	cholera	>250
Staphylococcus	31–62	typhus bacteria	>250
intestinal bacteria	>250		

Spectrum in vivo. Guinea pigs protected against experimental diphtheria infections by simultaneous administration of 0.1 ml of 0.25% solution; no protection if therapeutic dose is delayed, attributed to non-diffusibility of erythrin.

Erythrin applied to conjunctiva of monkeys, which had been traumatized and infected with diphtheria, led to disappearance of infection; similar results were obtained in preliminary trials on the oral-nasal cavity with respect to diphtheria.

BIBLIOGRAPHY

(1) Zil'ber, L. A., and Yakobson, L. M., "Erythrin, a new antibiotic substance of animal origin. I. Preparation of erythrin and its properties," *Zhur. Mikrobiol. Epidemiol. Immunobiol.,* **12,** 3 (1946); *Chem. Abs.,* **41,** 7,683g.

(2) Richter, V., "The chemistry of the carbon compounds," translated by Taylor, T. W. J., and Millidge, A. F., Vol. II, 382, Nordemann, New York, 1939.

EUMYCIN

First described by Johnson and Burdon in 1946; derived from a strain of *Bacillus subtilis.*

PRODUCTION

Fermentation. Surface growth on buffered yeast extract-proteose-peptone broth, at 30° C, optimum yields at 5 days' incubation.

Isolation. Culture liquor acidified, precipitate filtered and extracted with alcohol, alcohol extract evaporated, dissolved in NaOH at pH 7.0.

CHEMISTRY

Soluble in butyl alcohol, ethyl alcohol, acetone; insoluble in ether, amyl acetate; thermostable; unstable above pH 8.0.

BACTERIOLOGY

Most active against *Coryne. diphtheriae;* 0.005 mg per ml is bacteriostatic; at 0.1–0.3 mg/ml inhibits growth of *Trichophyton mentagrophytes, Microsporum gypseum, Epidermophyton floccosum* and related species of fungi, *Actinomyces, Myco. tuberculosis,* avian and human; less active against *Sporotrichum* and *Hormodendrum* species; slight activity against *Staph. aureus;* no activity against *Monilia, Cyptococcus,* and colon-typhoid bacilli.

PHARMACOLOGY

Low toxicity for mice.

BIBLIOGRAPHY

Johnson, E. A., and Burdon, K. L., "Eumycin, a new antibiotic active against pathogenic fungi and higher bacteria, including bacilli of tuberculosis and diphtheria," *J. Bact.*, **51**, 590 (1946).

EXPANSIN *See* CLAVACIN

FLAVICIDIN *See* PENICILLIN

FLAVACIN *See* PENICILLIN

FLAVATIN *See* PENICILLIN

FRUCTIGENIN *See* LATERITIIN GROUP

FUMIGACIN

First described by Waksman, Horning and Spencer in 1942[1]; *coincidentally observed by Wilkins and Harris in 1942*[2]; *described and named* HELVOLIC ACID by Chain, Florey, Jennings and Williams in 1943[3]; identified with fumigacin[8]; derived from *Aspergillus fumigatus*. This organism also produces SPINULOSIN, FUMIGATIN and GLIOTOXIN.[5, 8]

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l glucose 40, $NaNO_3$ 3.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; incubated at 28° C for 7–8 days; final pH 7.4; yield 100 *S. lutea* or 75 *B. mycoides* dilution units/ml.[4]

(B) g/l dextrose 40, $NaNO_3$ 3.0, NaH_2PO_4 1.0, KCl 0.5, $MgSO_4$ 0.5, $FeSO_4$ 0.01, pH 8; incubated at 24° C for 2–3 weeks; final pH 6.8.[3]

(C) Czapek-Dox; incubated at 24° C for 12–14 days; final pH 7.2.[6]

(D) Modified Czapek-Dox with brown sugar and blackstrap molasses; incubated at 23–25° C for 40 days.[7]

(E) Czapek-Dox with $MgSO_4$ 0.5 g/l, $FeSO_4$ 0.01 g/l in tap water; incubated at 28° C for 7 days; yield mg/l of fumigacin 24 (of fumigatin 103, of gliotoxin 69). Lower yields are obtained with distilled water, and with Mg and Fe added as chlorides.[5]

(F) Czapek-Dox with tap water; incubated at 24° C for 6–8 days; final pH 6.0; yield mg/l of fumigacin 3 (of gliotoxin 60). Addition of $ZnSO_4$ 1.0 mg/l suppressed production of fumigacin and promoted formation of fumigatin. With brown sugar in place of glucose in the culture medium, gliotoxin production is decreased, fumigacin production increased. With no inorganic S in the culture medium (Mg added as the chloride), little gliotoxin is produced, while fumigacin production is unchanged.[8]

Submerged growth in shaker flasks and aerated tanks on medium containing brown sugar 2%, KH_2PO_4 0.1%, $NaNO_3$ 0.6%, $MgSO_4$ 0.05%, $CaCO_3$ 1.5%; incubated for 6 days; yields of fumigacin and gliotoxin slightly better than with surface fermentation and in same relative proportion. (The effect of brown sugar on fumigacin-gliotoxin production in surface fermentation does not occur in submerged culture.)[8]

Isolation. (A) Culture filtrate activity adsorbed on activated carbon, the carbon cake dried and washed with ether, the activity eluted with chloroform; the chloroform extract evaporated to dryness; yield 60–156 mg/l.[1, 4] The carbon process yields in the active fraction a contaminating substance which impedes the crystallization of gliotoxin.[8] Yield mg/l fumigacin 15.1 (gliotoxin 27.1).[5]

(B) Culture filtrate acidified with HCl to pH 4.0, activity adsorbed on activated carbon, eluted with 80% acetone, acetone removed in vacuo, residue dissolved in chloroform.[3]

(C) Culture filtrate acidified to pH 2.0 with phosphoric acid and extracted with ether; ether extract concentrated, washed with saturated sodium bicarbonate and extracted with 6% sodium carbonate; the sodium carbonate solution containing fumigacin (the ether phase contains gliotoxin) is acidified and extracted with benzene.[8] Yield mg/l fumigacin 17.8 (fumigatin 39.3, gliotoxin 85.8).[5]

(D) Culture filtrate acidified to pH 3.6, extracted with chloroform, chloroform extract evaporated.[6]

(E) Crude fumigacin precipitated by acidification of the culture filtrate.[7]

Purification. (A) Crude fumigacin dissolved in warm alcohol, cooled and allowed to crystallize.[1, 4]

(B) Crystallized from benzene and recrystallized from methanol.[8]

(C) Dissolved in chloroform, passed through a magnesium oxide column and developed with 10% methanol in chloroform; activity eluted with boiling water, eluate acidified to pH 4, precipitating fumigacin; precipitate dissolved in acetone, water added to initiate precipitation and chilled, precipitating crystalline fumigacin; yield 4 mg/l; further purification by recrystallization from acetone-water.[3]

(D) Dissolved in chloroform and passed through an aluminum oxide column; the percolate concentrated and dissolved in ethanol; the ethanol solution concentrated and chilled, precipitating crystalline fumigacin (gliotoxin probably present in the mother liquor).[6]

(E) Dissolved in acetone, decolorized with activated carbon, recrystallized from methanol and ethanol.[7]

CHEMISTRY

Constitution. Proposed $C_{32}H_{44}O_8$[3, 9]; $C_{29}H_{38-40}O_7$[8]; $C_{32}H_{44}O_8$ or $C_{32}H_{42}O_8$[6]; by analysis C 69.55, 69.65, H 7.74, 7.90 [8]; C 69.20, H 7.85 [6]; M.W. 562 ± 12 (x-ray analysis) [9]; M.W. 510 (Rast), 562 (titration) [3]; M.W. 400–591 (titration) [8]; M.W. 533 (Rast).[6]

Properties. Four- and six-sided monoclinic crystals,[9] filamentous crystals,[8] fine long white needles,[1, 5] long, fine colorless needles [7]; m.p. 204.5–205.5° C (recryst. from pyridine), 209° C (recryst. from 70% acetic acid), 212° C (micro method),[3] 215–220° C (dec.),[8] 220° C,[6] 210.5–211° C [7]; $[\alpha]_D^{20} = -49.4°$ (3.5% in chloroform), $-47.3°$ (2.1% sodium salt in water) [3]; $[\alpha]_D^{25} = -132° \pm 2$ (0.41% in chloroform) [8]; $[\alpha]_{5,461}^{18} = -167°$ (2.37% in chloroform), $[\alpha]_{5,790}^{18} = -138°$ (2.37% in chloroform) [6]; ultraviolet absorption spectrum $E_{1\%}^{1cm} = 298$ at 234 mμ,[8] 300 at 230 mμ.[7]

Soluble in chloroform, acetone, ethyl acetate, benzene, pyridine, glacial acetic acid, dioxan, ether and warm alcohol; sparingly soluble in cold ethanol and methanol, petroleum ether and water [1,3,4,8]; sodium salt soluble in water, Ca, Mg, Ba, Fe, Cu, Zn, and Hg salts sparingly soluble.[3]

Reactions. Heat- and acid-stable, less stable in alkali [3,4,8]; yellow precipitate with ferric chloride; negative Fehling, ammoniacal silver nitrate [3]; negative ferric chloride, fuchsin sulfurous acid, Tollens, Molisch, Rosenheim, cholic acid, Jaffe-Tortelli, digitonin; positive bile acid, Liebermann-Burchard, Fehling [8]; reacts with 3 moles of alkali to give 2 moles of acetic acid; hydrogenation over PtO_2 in ethanol yields hexahydrofumigacin, in glacial acetic acid yields octahydrofumigacin.[7]

BACTERIOLOGY

Assay. Cylinder plate with *Staphylococcus* [3]; serial dilution with *Staph. aureus* [5,6,8]; heating at 100° C for 5 minutes in 1% sodium bicarbonate destroys gliotoxin but not fumigacin.[8]

Spectrum in vitro. Inhibition dilution (× 1,000) [3,4,5,6,10]:

Staph. aureus	80–2000	*N. intracellularis*	40
St. pyogenes	40	*S. typhi*	0.5
St. viridans	<160	*S. gaertneri*	25
D. pneumoniae III	10	*Sh. paradysenteriae*	<2.5
B. anthracis	160	*Sh. dysenteriae*	<2.5
C. diphtheriae	640	*Sh. sonnei*	<2.5
Cl. welchii	320	*E. coli*	0.5–1,200
Cl. edematiens	320	*Proteus*	0.5
Cl. septique	80	*Ps. aeruginosa*	0.5
Myco. smegmatis	<0.5	*Aero. aerogenes*	<40
S. schottmuelleri	<40	*B. mycoides*	1,250
Salmonella sp.	<40	*B. subtilis*	100–750
S. cholerae-suis	<40	*Sarcina lutea*	1,000–4,000
B. megatherium	1,250	*Myco. tuberculosis*	10–100
B. cereus	500	*Streptococcus* sp.	156

Inhibition concentration (γ/ml) [8]:

Staph. aureus	14–15	*St. hemolyticus*	5–25
Staph. albus	4–6	*St. viridans*	312
Staph. citreus	4	*B. anthracis*	6
Micro. tetragenus	60	*B. subtilis*	156

Mode of action. Bacteriostatic.[3]

Resistance. Staphylococcus and streptococcus resistance to fumigacin increased 250 times by successive transfers in fumigacin-containing media.[3]

Factors affecting activity. Slight decrease of activity by serum, pus, yeast extract, bacterial extract, liver extract, paraaminobenzoic acid, whole blood.[3]

Spectrum in vivo. In mice experimentally infected with staphylococcus and streptococcus, fumigacin prolonged survival time [3]; 2–4 mg subcutaneously afforded 50% protection against *St. hemolyticus.*[8]

PHARMACOLOGY

Toxicity. In mice, 16 mg intraperitoneally tolerated[5] (1.0 mg lethal dose[4] attributed to presence of gliotoxin)[6]; LD 50 intraperitoneally 400 mg/kg[8]; MLD intravenous 5–10 mg; 4 mg intraperitoneal caused liver necrosis; 20 mg oral was tolerated, repeated oral doses caused severe liver damage.[3]

On leucocytes, no effect at 1:1,600; on tissue culture no effect at 1:1,500.[3]

Blood levels, excretion. After intravenous administration, fumigacin appeared in the blood and urine.[3]

Absorption. Absorbed from gastrointestinal tract.[3]

Spinal fluid. None detected after parenteral administration.[3]

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FUMIGATIN

First described by Anslow and Raistrick in 1938,[1, 2, 3] derived from Aspergillus fumigatus and by synthesis.[4] This organism also produces SPINULOSIN, FUMIGACIN and GLIOTOXIN.

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l glucose 75, tartaric acid 4.0, ammonium tartrate 4.0, $(NH_4)_2HPO_4$ 0.6, K_2CO_3

0.6, $MgCO_3$ 0.4, $(NH_4)_2SO_4$ 0.25, $ZnSO_4 \cdot 7H_2O$ 0.07, $FeSO_4 \cdot 7H_2O$ 0.07; incubated at 24° C for 31–37 days.[1]

(B) Czapek-Dox with $MgSO_4$ 0.5, $FeSO_4$ 0.01 in tap water; incubated at 28° C for 7 days; yield mg/l of fumigatin 103 (of fumigacin 24, of gliotoxin 59).[5]

Isolation. Culture filtrate aerated (to convert to quinone form), acidified with HCl and extracted with chloroform; the chloroform extract evaporated *in vacuo* to dryness, the residue extracted with petroleum ether in a Soxhlet.[1]

Purification. Crystallized from petroleum ether.[1]

Synthesis. By treatment of 3:4:5 trimethoxybenzene with a solution of acetyl chloride and aluminum chloride in dry ether ⟶ 3-hydroxy-4:5-dimethoxy-2-acetyltoluene; the latter oxidized with hydrogen peroxide in dilute alkali ⟶ 2:3-dihydroxy-4:5-dimethoxytoluene; the latter oxidized in dilute solution with ferric chloride under petroleum ether ⟶ fumigatin.[4]

CHEMISTRY

Constitution. 3-hydroxy-4-methoxy-2:5 toluquinone.[1, 2, 3]

Properties. Maroon-colored crystals; m.p. 116° C; sublimes in high vacuum; soluble in acetone, ether, chloroform, benzene, ethyl acetate, alcohol; fairly soluble in water; slightly soluble in petroleum ether.[1]

Reactions. Unstable in aqueous solution.[6]

Derivatives. By reduction, yielded corresponding quinol, 3-hydroxy-4-methoxytoluquinone [1]; the latter also found in culture liquor.[2] Converted into SPINULOSIN by Thiele-Winter reaction forming tetraacetate; the latter hydrolyzed to tetrahydroxymethoxytoluene, followed by oxidation by air in alkaline solution to yield spinulosin.[1]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus.*[5]

Spectrum in vitro. Inhibition dilution (× 1,000) [5, 6]:

E. coli	1.2–12	*B. subtilis*	40
Staph. aureus	<1.2–200	*B. anthracis*	33–50
Staph. albus	20–33	*V. comma*	100
St. viridans	1.2–20	*S. typhi-murium*	<2
		Sar. lutea	100

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FUSCIN

First described by Michael in 1948 [1]; derived from *Oidiodendron fuscum.*

PRODUCTION

Fermentation. Surface growth on Czapek-Dox; incubated at 24° C for 21–30 days; yield 0.3 g/l.[1, 2]

Isolation. Fuscin is present in the culture liquor in the colorless dihydro form; converted to crude fuscin by aeration at pH over 9.0 in the presence of ferrous sulfate.[1, 2]

Purification. Crude fuscin extracted with boiling ethanol, the alcohol extract cooled and fuscin allowed to crystallize.[2]

CHEMISTRY

Constitution. Quinoid structure; proposed $C_{15}H_{16}O_5$, C 65.2, H 5.8, one active hydrogen 0.36, 2 $C—CH_3$ 10.9, M.W. 276; found C 65.0, 64.9, H 5.9, 5.7, active hydrogen in pyridine 0.38, $C—CH_3$ 10.2, M.W. 276.[2]

Properties. Shiny orange diamond-shaped plates; m.p. 230° C; sublimes in high vacuum; soluble in chloroform, acetone, hot ethanol, ethyl acetate, acetic acid; moderately soluble in ether, benzene; slightly soluble in cold ethanol; almost insoluble in petroleum ether and water; optically inactive.[1, 2]

Reactions. Purple color in alkali and aqueous pyridine; red-purple with ferric chloride.[1, 2]

Derivatives. Stable crystalline compound with thioglycollic acid; colorless needles, m.p. 193° C; antibiotically active.[2, 3]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus.*[1]

Spectrum in vitro. Inhibition dilution (× 1,000) [3]:

Staph. aureus	80–1,280	*S. enteriditis*	<5
Staph. albus	80	*S. paratyphi*	<5
Staph. citreus	1,280	*S. typhi*	5
St. pyogenes	640	*S. typhi-murium*	10
St. viridans	640	*Sh. flexneri*	<5
B. anthracis	320–1,280	*Sh. sonnei*	<5
B. subtilis	80	*Sh. shigae*	10
C. diphth. gravis	160	*Myco. phlei*	10
C. diphth. intermed.	320	*Myco. smegmatis*	20
C. diphth. mitis	320	*Pr. vulgaris*	5
E. coli	5	*V. comma*	80

Fuscin-thioglycollic acid, inhibition dilution (× 1,000)[3]:

Staph. aureus	40–160	*E. coli*	<5
B. subtilis	40	*Pr. vulgaris*	5
B. anthracis	640	*V. comma*	20
C. diphth. mitis	640		

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GENTISYL ALCOHOL

First described by Birkinshaw, Bracker and Raistrick in 1943; derived from *Penicillium patulum* Bainier,[1] from *P. divergens* Bainier,[4] from a *Penicillium* sp.[2]; also from *P. urticae* as a quinhydrone with hydroxymethyl-*p*-benzoquinone.[5]

PRODUCTION

Fermentation. Surface culture with *P. patulum* Bainier on Raulin-Thom solution at 24° C for 14 days[1]; with *Penicillium* sp. on medium containing g/l: sodium nitrate 3, KH_2PO_4 1, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, glucose 40, $FeSO_4 \cdot 7H_2O$ 0.001, $ZnSO_4$ 10^{-5}–10^{-7} mole; yield 2 g/l.[1]

Isolation. *Of gentisyl alcohol.* (A) Culture filtrate evaporated in vacuo, extracted with ethyl acetate, extract dried with sodium sulfate, solvent removed in vacuo, residual oil dissolved in ether, passed through a column[3] to separate patulin; patulin-free ether evaporated, residual oil dissolved in ether, and hot chloroform added, precipitating crude crystalline product[1]; yield 0.6 g/l.[3]

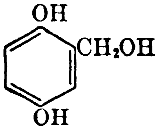
(B) Culture filtrate concentrated in vacuo, syrupy residue distributed in a column between water and ether, ether extracts containing gentisyl alcohol evaporated, crude residue dissolved in methyl alcohol diluted with chloroform, precipitating pure crystalline product; yield 1–1.5 g/l.[2]

Isolation of quinhydrone with hydroxymethyl-p-benzoquinone. Culture filtrate of *P. urticae* acidified to pH 4, saturated with sodium chloride, extracted with acetic acid ester, the extract shaken with half-saturated sodium bicarbonate solution, dried over sodium sulfate, concentrated in vacuo to a dark violet residue; yield 0.9 g/l [5]; also prepared by recrystallization of a synthetic mixture of 3 moles of gentisyl alcohol and one mole of hydroxymethyl-*p*-benzoquinone from ether-petroleum ether.[5]

Purification. *Of gentisyl alcohol:* by recrystallization from hot chloroform [1]; *of quinhydrone with hydroxymethyl-p-benzoquinone:* by recrystallization from acetic ester-petroleum ether.[5]

CHEMISTRY

Constitution of gentisyl alcohol. $C_7H_8O_3$, structure confirmed by synthesis from gentisyl aldehyde [1]:



Properties of gentisyl alcohol. Colorless needles, m.p. 100° C, (recrystallized from chloroform containing 2% methanol), m.p. 101° C, sublimes in vacuo at bath temperature 70–80°; slightly decolorized on exposure to air.[1, 2] Soluble in water, ethanol, ether; slightly soluble in benzene, chloroform; insoluble in light petroleum.[1]

Reactions. Aqueous solution neutral to litmus; decolorizes permanganate; reduces silver nitrate; blue color with aqueous ferric chloride changing to yellow, reacts with aqueous iodine; yellow color with 2*N* NaOH, darkens to reddish brown; gives white amorphous precipitate when heated with 2*N* sulfuric acid [1, 2]; molecular compound, a quinhydrone, formed from three moles of gentisyl alcohol and one mole of hydroxymethyl-*p*-benzoquinone $(C_7H_8O_3)_3$.[5]

Properties of quinhydrone with hydroxymethyl-*p*-benzoquinone. Small needles recrystallized from acetic ester-petroleum ether, m.p. 86–89° C not sharp (corr.); soluble in water, alcohol, acetone, acetic ester, ether; slightly soluble in benzene, chloroform, petroleum ether; aqueous solutions give evanescent green-blue color with ferric chloride, intense green color with sodium hydroxide, sodium carbonate or ammonia, changing to dark-brown; rapidly decolorizes potassium permanganate and iodine.[5]

BACTERIOLOGY

Gentisyl alcohol almost completely inhibited growth of *Staph. aureus* at 1:500.[1]

The quinhydrone with hydroxymethyl-*p*-benzoquinone inhibited *Staph. aureus* at 1:5,000.[5]

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GEODIN

First described by Raistrick and Smith in 1935 [1]; derived from *Aspergillus terreus*.

PRODUCTION

Fermentation. Surface growth in medium containing g/l glucose 50, $NaNO_3$ 2.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, incubated at 24° C for 26 days.[2]

Isolation. (A) Culture filtrate acidified, flocculent brown precipitate filtered, washed with water, dried in vacuo; yield 31 g from 28 liters; precipitate extracted with boiling chloroform, filtered hot, diluted with ether and cooled, precipitating crystalline geodin.[2] (B) Culture filtrate adjusted to pH 6.6, stirred with activated carbon; carbon filtered and dried at room temperature; extracted with methanol; methanol extract concentrated, precipitating crystalline geodin; yield 0.7 g from 3 liters.[7]

Purification. By recrystallization from chloroform-ether, ethyl acetate, methanol, aqueous acetone.[2, 7]

CHEMISTRY

Constitution. Proposed $C_7H_{12}O_7Cl_2$; M.W. 380 (cryoscopic in dioxan); titration equivalent 198,199.[2, 3, 4, 5, 7]

Properties. Rosettes of fine pale-brown needles from chloroform-ether, m.p. 225–230° C (dec.); pale yellow fine needles recrystallized from chloroform-ether, m.p. 235° (dec.); fine brown needles recrystallized from ethyl acetate, m.p. 235° (dec.); pale yellow microcrystals by sublimation, m.p. 235° C (dec.) [2]; recrystallized from methanol or aqueous acetone 227–230° C.[7] Insoluble in water, petroleum ether; slightly soluble in benzene or ether; soluble in alcohol, ethyl acetate, chloroform, acetone, dioxan and sodium bicarbonate; $[\alpha]_{5,461}^{20°} = +179°$, $[\alpha]_{5,790}^{20°} = +149°$ in chloroform (c = 0.8); neutral solution of disodium salt $[\alpha]_{5,461}^{20°} = +72.4°$, $[\alpha]_{5,790}^{20°} = +65.6°$.[2]

Reactions. Green to brown color in alcohol solution with ferric chloride; brown-gray precipitate with ferric chloride and neutral sodium salt.[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 1,000)[6]:

B. subtilis	32–1,000	*E. coli*	40
Staph. aureus (9 strains)	8–2,000	*Ps. fluorescens*	20
Staph. citreus	128	*Strep. viridans*	8
Strep. pyogenes	128	*Staph. albus*	8
B. anthracis	32–128	*Myco. smegmatis*	4–20
C. diphtheriae	23–128	*V. cholera*	2
Mycobacterium sp.	32	*Shig. dysenteriae*	1

Factors affecting activity. Activity lowered by cystein and by serum.[7]

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GIGANTIC ACID *See* PENICILLIN

GLADIOLIC ACID

First observed by Wilkins and Harris in 1943 [1]; isolated and named by Brian, Curtis, Grove, Hemming and McGowan in 1946 [3]; derived from *Penicillium gladioli.*

PRODUCTION

Fermentation. Surface culture on Raulin-Thom medium (7.5% dextrose), yield 32 *Botrytis allii* units/ml.

Isolation. Culture filtrate acidified to pH 4.0, treated with activated carbon; carbon cake dried at room temperature, extracted with ether; ether extract evaporated to a brown or yellow pasty material.

Purification. Recrystallization from water and drying in vacuo over sulfuric acid; yield up to 300 mg/l.[3]

CHEMISTRY

Constitution. Proposed $C_{11}H_{10}O_5$ M.W. 222.1 (by analysis), 212 (Rast); (titration equivalent 228, 207); proposed structure: methoxymethyl-2-carboxyphenyl glyoxal.[3]

Properties. Long, colorless, silky needles; m.p. 160° C; monobasic acid; with strong ammonia deep green color changing to red after 12 hours and then to orange several days later; aqueous solutions in McIlvaine's buffer stable for 10 days at 25° C at pH 3–8.[3]

BACTERIOLOGY

Assay. By inhibition of germination of *Botrytis allii* conidia: 2 mg/ml at pH 3.5, 100 mg/ml at pH 7.

Spectrum in vitro. At pH 7 inhibition of *Staph. aureus* at 250 g/ml; ineffective on *S. typhi* or *E. coli* at 500 g/ml; has marked fungistatic properties.[3]

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GLIOTOXIN

First described by Weindling in 1932 *et seq.*[1-4]; named from *Gliocladium fimbricatum*, the identification assigned to the gliotoxin-producing organism,[4] later indicated to be *Trichoderma viride*.[5] Also described as "ASPERGILLIN," [6] closely resembling or identical with gliotoxin.[7] Derived from *T. viride* (*T. lignorum*),[1, 5] *Aspergillus fumigatus*,[7, 8] *Penicillium obscurum*,[9] *P. jenseni* [22] and *Penicillium* sp.[15] *A. fumigatus* also produces SPINULOSIN, FUMIGACIN and FUMIGATIN.[8, 9, 10]

PRODUCTION

Fermentation. With *T. viride:* (A) Surface growth on culture medium containing g/l: dextrose 25, ammonium tartrate 2, KH_2PO_4 2, $MgSO_4$ 1.0, ferrous sulfate 0.01, pH 3.5; incubated at 25° C for 4 days; yield approximately 2,000 *Botrytis allii* units/ml.[11] (B) Percolation through blotting paper column; yield approximately 1.5 g per week from 25 l of culture liquor.[11] (C) Submerged growth in shaker flasks on medium containing ammonium tartrate as nitrogen source pH 3–4; incubated for 2 days; yield 70 mg/l.[12] (D) Submerged growth in shaker flasks on medium containing g/l cane sugar 15, ammonium sulfate 1.7, K_2HPO_4 0.8, $MgSO_4$ 0.4, $FeCl_3$ 0.008, peptone 0.017, pH 3.0–3.5; yield 50 mg/l.[13]

With *A. fumigatus:* (A) Surface growth on Czapek-Dox incubated at 24–28° C for 5–8 days [7, 8, 10]; yield mg/l gliotoxin 80,[7] gliotoxin 60 (fumigacin 3),[8] gliotoxin 69 (fumigacin 24, fumigatin 103).[10] Gliotoxin production reduced by addition of brown sugar to or elimination of sulfate from culture medium.[8, 10] (B) Submerged growth in shaker flasks and in aerated tanks on medium containing brown sugar 2%, KH_2PO_4 0.1%, $NaNO_3$ 0.6%, $MgSO_4$ 0.05%, $CaCO_3$ 1.5%; incubated for 6 days; yield of gliotoxin (and fumigacin) slightly better than with surface fermentation and in same relative proportion. (The effect of brown sugar on gliotoxin-fumigacin production in surface fermentation does not occur in submerged culture).[8]

Isolation. (A) Culture filtrate extracted with chloroform, the chloroform extract distilled, the residue taken up in hot benzene or 95% ethanol, cooled and allowed to crystallize.[3, 12] (B) Chloroform extract of culture filtrate evaporated, the residue dissolved in methanol, the solution treated with activated carbon and allowed to crystallize.[13] (C) Culture filtrate acidified to pH 2 with phosphoric acid and extracted with ether; the ether extract concentrated, washed with saturated sodium bicarbonate and extracted with 6% aqueous sodium carbonate (removing fumigacin); the ether phase evaporated, the residue crystallized from ethanol.[8] (D) Culture filtrate extracted with ether; the ether extract distilled, the residue dissolved in boiling alcohol, chilled and allowed to crystallize.[7] (E) Culture filtrate at pH 2 extracted with benzene, the benzene extract concentrated and petroleum ether added precipitating crude gliotoxin.[9]

Purification. Recrystallization from ethanol, methanol, ethyl acetate or benzene.[7, 8, 9, 12, 13]

CHEMISTRY

Constitution. Proposed $C_{13}H_{14}N_2S_2O_4$, C 47.85, H 4.32, N 8.59, S 19.6, M.W. 326.[7, 8, 13, 14] By analysis:

C	H	N	S
48.42	4.47	8.38	19.06[7]
48.55	4.36	8.48	19.26[7]
48.68	4.24	8.40	19.36[8]
48.77	4.56[8]		

M.W. 347, 213, 281 (cryo. diphenylamine), 263 (chloroform), 288 (acetone), 314 (cryo. ethyl bromide).[12, 13]

Previously proposed $C_{14}H_{16}N_2S_2O_4$, C 49.38, H 4.73, N 8.23, S 18.82, M.W. 340. By analysis C 47.75, 48.08, H 4.36, 4.43, N 8.25, 8.27, S 19.4.[12]

Proposed structures and derivatives bearing on structure.[16-19]

Properties. Colorless elongated plates or needles[7, 10]; $\alpha = 1.644 \pm 0.001$, $\beta = 1.658 \pm 0.001$, $\beta' = 1.6554 \pm 0.002$, $\gamma = 1.7075 \pm 0.0004$[13]; m.p. 215–220° C (dec.),[7] 195° C,[8, 10] 192–193° C (corr.),[9] 219–222° C,[12] 221° C.[13]

Solubility mg/ml at 30° C: tert. BuOH 6.2, chloroform 28, HCl 0.1*N* 0.24, water 0.07; at 7° C: acetic acid 12, acetone 9, acetonitrile 10.2, benzene 5.5, carbon tetrachloride 0.8, chloroform 20, dioxane 73 (slowly decomposes), dimethyl formamide 17, ethyl acetate 8.5, ethanol 4.7, methanol 1.4, pyridine 77.[13]

Optical activity: $[\alpha]_D^{20} = -256$,[7] $[\alpha]_D^{22} = -245°$ (chloroform),[8] $[\alpha]_D^{19°} = -239°$ (chloroform)[12]; $[\alpha]_D^{25} = -290° \pm 10°$ (0.078 ethanol), $-270° \pm 10°$ (1.70 pyridine), $-255° \pm 15°$ (0.103 chloroform); in ethanol with 0.1*N* NaOH, $[\alpha]_D^{25} = +111°$ changing to +80° at 48 hours and to 0° at 5 days.[13]

Ultraviolet maxima at 270 and 450 mμ, minima at 245, 350 mμ.[8]

Reactions. Stable in acid, unstable in alkaline solution[3, 7, 11]; readily oxidized with sulfur, forming sulfates; reduced to yield hydrogen sulfide[16]; in boiling alkali gave black precipitate with lead acetate.[12]

BACTERIOLOGY

Assay. Serial dilution[6, 7]; differential serial dilution with *Staph. aureus* (heating at 100° C for 5 minutes in 1% sodium bicarbonate to destroy gliotoxin but not fumigacin)[8]; serial dilution with *Rhizoctonia solani*, inhibited at 1:300,000 of crystalline gliotoxin[12]; inhibition of spore germination, *Botrytis allii* spores inhibited at 3.75 γ/ml.[11]

Spectrum in vitro: Inhibition concentration in agar media (γ/ml)[13]:

Staph. aureus	0.5	*Eb. typhi*	2.5
Staph. albus	1–2.5	*E. coli*	5–10
St. viridans	0.5	*S. schottmuelleri*	10
N. catarrhalis	0–0.2	*S. paratyphi*	2.5–5
H. pertussis	1–2.5	*Epidermophyton* sp.	0.8

Inhibition concentration in broth (γ/ml) [11, 13, 22]:

St. pyogenes	0.936	*Aero. aerogenes*	4.8
Staph. aureus	0.234	*K. pneumoniae*	3.9
Pneumococcus III	0.234	*E. coli*	12.7
Pneumococcus I	0.312	*Epidermophyton* sp.	15.6
S. enteriditis	3.9	*Micro. lysodeikticus*	0.15

Boletus bovinus	0–5	*Mycorrhizum radicis nigro-strigosum*	5–10
Boletus elegans	10–20	*Mycorrhizum radicis atrovirens*	5–10
Rhizoctonia sp.	5–10	*Phoma radicis callunae*	20–40

Inhibition dilution (× 10,000) [7, 10, 13]:

C. diph. gravis	512–2,048	*Staph. aureus*	1–16
S. pneumoniae	256–2,048	*B. subtilis*	32
N. intracellularis	2,048	*B. flexneri*	32
St. pyogenes	256	*S. typhi*	4
St. viridans	128	*S. typhimurium*	2
Cl. tetani	128	*Pr. vulgaris*	<1
Cl. welchii	32	*Ps. aeruginosa*	<1
Br. abortus	64	*H. influenzae*	<1

Ps. fluorescens	<10	*Myco. synxanthum*	1–10
Phyto. michiganensis	ca. 10	*Lacto. casei*	<0.1
Sar. lutea	<10	*St. lactis*	0.1–1.0
B. mesentericus	1–10	Yeast	32

E. coli	0.6	*B. subtilis*	75
Staph. aureus	150	*Sar. lutea*	200

Gliotoxin is active against *P. italicum*, *Rhizopus* sp., *Fomes annosus*, *Blastomycoides dermatitidis;* not against *A. niger*, *Trichoderma* sp., *P. digitatum.*[13]

Factors affecting activity. Antagonized by cysteine,[20] oxyhemoglobin; slight reduction of activity by rabbit serum.[7]

Resistance. *Staph. aureus* increased in resistance 12–13 times, *S. typhi* 24 and *Cl. welchii* 256 times.[7]

Spectrum in vivo. In mice, no protection against streptococcus by 0.5 mg subcutaneously.[7] Some protection and no phytocidal effect by treatment with gliotoxin of oats, barley and wheat against *Helminthosporium avenae* (oats), *Ustilego hordei* (barley) and *Tilletia caries* (wheat).[11]

PHARMACOLOGY

Toxicity. Lethal dose mg/kg in mice and rats, intraperitoneal and oral, 50–65; in rabbits, intravenous, 45.[7, 13]

MISCELLANEOUS

Lymphosarcoma, sarcoma and carcinoma showed growth inhibition in tissue culture with 1:10–1:80 of crude culture filtrate. *In vivo* results with mice were not encouraging.[21]

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GLUTINOSIN

First described by Brian and McGowan in 1946[1]; derived from *Metarrhizium glutinosum*.

PRODUCTION

Fermentation. Surface growth on medium containing (A) Raulin-Thom with crude glucose; incubated at 25° C for 8 days; yield 256 *B. allii* units/ml. (B) g/l glucose (crude) 50, phosphoric acid 0.75, malic acid 10.0, magnesium sulfate 0.5, trace elements, pH 4.0; yield 100 mg/l.[2]

Isolation. (A) Culture filtrate extracted with ether, petroleum ether, *n*-butanol or benzene and the extract evaporated to dryness. (B) Activity of culture filtrate adsorbed on activated carbon, eluted with hot benzene.[1, 2]

Purification. Recrystallization from hot ethanol.[1, 2]

CHEMISTRY

Constitution. Proposed, $C_{48}H_{60}O_{16}$ by analysis, M.W. found 920, 892.7, 755 (Rast).[1]

Properties. Thin colorless plates; darken without melting at 300° C; $[\alpha]_D^{20}$ = ca. + 54° (0.2% in benzene).[1]

Reactions. Stable in solution at pH 2.9–7.6.[1]

BACTERIOLOGY

Assay. Inhibition of spore germination of *Botrytis allii;* one unit equals 0.2 γ/ml of glutinosin.[1, 2]

Spectrum in vitro. No inhibition of several bacterial species at 50–100 γ/ml.[1, 2]

Concentration for growth inhibition (γ/ml)[2] :

Mucor mucedo	1–5	*Aspergillus oryzae*	100–>100
Mucor erectus	>100	*Botrytis allii*	5–25
Phytophthora cryptagea	25–100	*Fusarium coeruleum*	>100
Phytophthora palmivora	5–25	*Fusarium graminearum*	>100
Thamnidium elegans	5–25	*Metarrhizium glutinosum*	>100
Byssochlamys fulva	<1–1	*Monilia sitophila*	100–>100
Chaetomium elatum	5–25	*Penicillium digitatum*	1–5
Chaetomium globosum	>100	*Penicillium expansum*	100–>100
Endomycopsis albicans	25–100	*Penicillium gladioli*	25–100
Gibberella saubinetti	>100	*Phoma betae*	<1–1

Melanospora pampeana	>100	*Stachybotrys atra*	>100
Neurospora crassa	<1-1	*Torulopsis utilis*	100->100
Saccharomyces cerevisiae	5-25	*Trichoderme viride*	>100
Stereum purpureum	<1-1	*Trichothecium roseum*	>100
Hydnum coralloides	<1	*Verticillium cinnabarinum*	>100
Aspergillus niger	>100	*Verticillium dahliae*	100->100

Concentration for spore germination inhibition (γ/ml)[2]:

Mucor mucedo	1.6	*Penicillium expansum*	25->50
Syncephalastrum racemosum	12.5	*Penicillium gladioli*	3.1
Thamnidium elegans	0.4	*Stachybotrys atra*	1.6
Aspergillus niger	25	*Stemphylium* sp.	50
Fusarium coeruleum	0.8-3.1	*Trichoderma viride*	>50
Fusarium graminearum	3.1	*Trichothecium roseum*	0.8
Penicillium digitatum	1.0-3.1	*Verticillium cinnabarinum*	25.0

PHARMACOLOGY

A volatile component of the fermented culture liquor, not glutinosin but extracted with it, caused on contact a severe dermatitis of the skin and severe reactions in the nose and throat.[1, 2]

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GRAMICIDIN See TYROTHRICIN

GRAMICIDIN S See TYROTHRICIN

GRAMIDINIC ACID See TYROTHRICIN

GRAMINIC ACID See TYROTHRICIN

GRISEIN

First described by Reynolds, Schatz and Waksman[1]; derived from *Streptomyces griseus.*

PRODUCTION

Fermentation. In surface and submerged culture, on medium containing peptone 5 g, neutralized corn steep liquor 10 ml, NaCl 5 g; also in synthetic medium with ammonium citrate as best nitrogen source; yields greatly increased by addition of Fe as ferrous sulfate 100 mg/l, or as ferric ammonium citrate or peptonized iron on both synthetic and organic media; yields retarded by glucose; fermentation time: 6–12 days in surface culture, 4–6 days in submerged culture at 28° C; yield in a typical experiment, 700 *E. coli* units per ml in submerged culture with a synthetic medium including Fe; "2,200 units per ml or even higher" in other experiments.[1, 2]

Isolation. Activity of fermented liquor filtrate adsorbed on activated carbon, eluted with 95% ethanol, eluate concentrated in vacuo to a syrup, active material precipitated with absolute methanol followed by acetone, precipitate washed with ether and desiccated in vacuo; yield 14.5% of activity of culture liquor with potency of 430 *E. coli* units per mg.[2]

CHEMISTRY

Soluble in water; slightly soluble in acetone, 95% ethanol; insoluble in ether, chloroform, absolute acetone, absolute ethanol, absolute ether; thermostable for 10 minutes at 100° C, stable for short periods at pH 4.0–10.5; not absorbed by "Filtercel," alumina at pH 4.5, Berkefeld filter.[1, 2]

BACTERIOLOGY

Assay. Serial dilution and plate methods, using a streptomycin-resistant strain of *E. coli* as test organism.[1, 2]

Spectrum in vitro. Inhibition by units per mg of "a potent preparation of grisein" [2]:

E. coli	1,800	*S. alkalescens*	1,800
S. pullorum	30,000	*B. megatherium*	1,000
Micro. lysodeikticus	30,000	*B. cereus*	1,000
S. dysenteriae	18,000	*B. subtilis*	300
S. paradysenteriae	18,000	*S. marcescens*	<100
Staph. aureus	3,000	*Ps. aeruginosa*	<100

Ps. fluorescens, Aero. aerogenes, K. pneumoniae, Pr. vulgaris, Myco. tuberculosis, Myco. phlei, Sar. lutea < 30.

Resistance. Several susceptible bacteria show rapid development of resistant strains.

Factors affecting activity. Grisein activity is lowered by increased acidity of the assay substrate; unaffected by cysteine, sodium thioglycolate, hydrazine, hydroxylamine, semicarbazide; inactivated by ferric iron in assay substrate (0.0004*M* ferric ion completely nullifies grisein activity on *E. coli*).[2]

Spectrum in vivo. Mouse protection against *Salmonella schottmulleri* and *Staph. aureus* by 800–1,600 units.[1, 2]

PHARMACOLOGY

Experimental animals tolerated 500,000 units per kg of grisein; activity detected in urine.[1]

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GRISEOFULVIN

First isolated and named by Oxford, Raistrick and Simonart in 1939 [1]; described as an antibiotic and named CURLING FACTOR (from its effect in causing distortion or "curling" in growing mold hyphae) by Brian, Curtis and Hemming in 1946 [2]; curling factor identified as griseofulvin [3, 4]; derived from *Penicillium griseofulvum*,[1] *P. janczewski* [2] and *P. patulum*.[4]

PRODUCTION

Fermentation. With *P. griseofulvum*, surface growth on medium containing g/l glucose 80, $NaNO_3$ 2.5, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.02; incubated at 30° C for 65–85 days [1]; yield 192 mg from 11.5 g of mycelium equivalent one liter of culture medium.[1, 4]

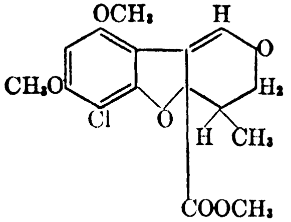
With *P. janczewskii*, surface growth on Weindling or Raulin-Thom medium; best yields with g/l dextrose 75, KNO_3 2.3, KH_2PO_4 1.0, $MgSO_4$ hydrated 0.5, trace elements; increased yields with ammonium ion as nitrogen source and with chlorides, *e.g.*, KCl 0.005–0.01%; incubated at 25° C; yield up to 150–170 mg/l from culture liquor, 240 mg from 17 g of mycelium equivalent one liter of culture liquor.[2, 4]

Isolation. (A) Mycelium extracted in Soxhlet with petroleum ether, extract discarded; mycelium then extracted with ether; the ether extract evaporated to dryness.[1] (B) Culture filtrate extracted with chloroform, or activity adsorbed on activated carbon and eluted with chloroform or ether; extract or eluate evaporated to dryness.[2]

Purification. (A) Residue extracted with hot benzene, the benzene cooled and precipitated impurities removed; the benzene extract then concentrated and allowed to crystallize; recrystallized from ethanol.[1] (B) Recrystallization from hot ethanol or from aqueous dioxan.[2] (C) Recrystallization from 1,4-dioxan (or *i*-butanol or aqueous tetrahydrofurfuryl alcohol) followed by crystallization from ethanol.[5]

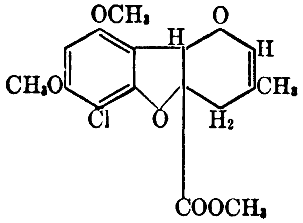
CHEMISTRY

Constitution. Proposed $C_{17}H_{17}O_6Cl$,[1]



(ref (1.))

or



(ref. (3))

Requires: C 57.86, H 4.85, Cl 10.06, 3 CH_3O 26.4, M.W. 352.5; found C 58.05, 57.86, H 4.93, 4.90, Cl 9.98, 10.09, CH_3O 26.29, N nil, M.W. 309 (cryo. dioxane), 348 (cryo. camphor) [1]; C 59.3, 59.2, 58.5, H 4.9, 5.0, 4.9, M.W. 398, 387, 378 (Rast).[5]

Properties. Massive colorless rhombic crystals from alcohol,[1] octahedral crystals [5]; m.p. 218–219° C,[1] 220° C [5]; soluble in acetic acid, 1,4-dioxan, benzene, ether, ethanol [5]; sparingly soluble in chloroform, ethyl acetate, benzene, toluene, ethanol, acetone, dioxan, ligroin [1, 5]; insoluble in water [1]; $[\alpha]^{19°}_{5790} = +354°$, $[\alpha]^{19°}_{5461} = 417$ ($c = 1.0127$ acetone),[1] $[\alpha]^{17}_{D} = +370°$ (2% in chloroform).[5]

Reactions. Thermostable [2]; negative with ferric chloride, HBr, alkaline sodium nitroprusside, pyridine; yellow color with sulfuric and with nitric acid; reacted with phenylhydrazine and 2,4-dinitrophenylhydrazine but not with diazomethane or semicarbazide.[1]

BACTERIOLOGY

Assay. Serial dilution with *Botrytis allii* spores; 0.2 γ/ml causes distortion or "curling" of growing hyphae and stunting of germ tubes.[2]

Spectrum in vitro. Growth inhibition in all *Zygomycetes*, *Ascomycetes* (except *Sacch. cerevisiae*), *Basidiomycetes* (except *Coniophora cerebella*) and *Fungi imperfecti* (except *Torulopsis utilis*) tested, at 20 γ/ml. No activity against a number of *Oomycetes*, bacterial and actinomycete species.[6]

PHARMACOLOGY

Phytotoxicity. Toxic to angiosperm seeds at 25 γ/ml.[6]

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GRISIC ACID

First described by Robbins, Kavanagh and Hervey in 1945; derived from a *Basidiomycetes* sp.

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HELVOLIC ACID See FUMIGACIN

HEMIPYOCYANIN See PYO COMPOUNDS

HERQUEIN

First described by Burton in 1949; derived from *Penicillium herquei.*

PRODUCTION

Fermentation. Surface growth on medium containing g/l glucose 40, $NaNO_3$ 3, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; incubated 8–15 days.

Isolation. Culture liquor acidified to pH 2.0, the precipitate removed, suspended in water and extracted with chloroform; chloroform extract shaken with water at pH 7.5, the aqueous phase removed and acidified with HCl, precipitating herquein as yellow microcrystals.

Purification. Recrystallization from aqueous alcohol or benzene.

CHEMISTRY

Constitution. Proposed $C_{19}H_{20}O_8$, C 60.6, H 5.2; found C 59.84, H 5.42, OCH_3 5.33, $(C)CH_3$ 10.5.

Properties. Yellow-brown crystals; m.p. 129° C (dec.); soluble in ethanol, chloroform; sparingly soluble in water, ether, carbon tetrachloride, benzene; insoluble in petroleum ether.

Reactions. Precipitated by lead acetate.

BACTERIOLOGY

Inhibited *V. comma* and *Staph. aureus* at 1:2,500; also active against *Sh. dysenteriae*, *M. phlei*, *St. pyogenes*, *Ps. aeruginosa* and *Staph. albus*.

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HIRSUTIC ACID

First described by Heatley, Jennings and Florey in 1947; derived from *Stereum hirsutum*.

Hirsutic acid is a generic term for a series of substances, of which Hirsutic Acid C is the precursor. Hirsutic Acid C is produced by the fungus early in its life cycle and is biologically inactive. By continued growth and metabolism, the fungus converts Hirsutic Acid C into the biologically active Hirsutic Acid N. The same conversion may be accomplished by incubation of Hirsutic Acid C with washed mycelium or mycelial extract. By treatment of Hirsutic Acid C with dilute alkali, antibiotically active Hirsutic Acid A is formed. Other derivatives have been described: Hirsutic Acid X, 1, 2, 3, 4.

PRODUCTION

Fermentation. Surface culture on 2% malt extract in tap water at 24° C, maximum yield at 10–11 days.

Isolation. *Hirsutic Acid C.* Culture medium acidified to pH 2.0, extracted with amyl acetate, amyl acetate extract evaporated in vacuo to a thin syrup, chilled, crystalline Hirsutic Acid C removed.

Hirsutic Acid N. (A) Mother liquor from Hirsutic Acid C crystallization diluted with ether, passed through an alumina column, percolate concentrated by distillation, extracted with dilute aqueous sodium hydroxide at pH 6.0–6.5, aqueous extract acidified to pH 2.0, extracted with ether; (B) Intact or macerated mycelium, 9 or more days old, washed with distilled water, incubated for about 5 hours at 37° C with neutralized Hirsutic Acid C (mycelial conversion activity destroyed by boiling, not affected by chloroform, stable for 6 months at 4° C).

Hirsutic Acid A. Incubation of Hirsutic Acid C in $N/10$ sodium hydroxide at 20–24° C for 30 minutes, acidified to pH 2.0, chilled, unchanged Hirsutic Acid C filtered off.

Purification. *Hirsutic Acid C.* Recrystallized from alcohol or aqueous alcohol.

Hirsutic Acid N. Crude ether extract passed through silica-phosphate buffer (pH 7.0) column, washed with ether, ether percolates concentrated by distillation, extracted into water at pH 6.5–7.0, aqueous extracts treated with activated carbon, acidified, extracted into ether; ether extract passed through an alumina column; active band showing purple fluorescence under ultraviolet eluted with phosphate buffer, acidified, extracted into ether; ether extract evaporated to a clear transparent colorless glaze.

Hirsutic Acid A. Extracted into ether, some purification by passage through an alumina column; ether solution evaporated to a colorless brittle glaze.

CHEMISTRY

Constitution. *Hirsutic Acid C.* Proposed $C_{15}H_{20}O_4$, with calculated C 68.2, H 7.57; found C 68.3, H 7.47; M.W. calc., 264; found 260–264 by titration; 268 ± 6 by x-ray crystallography.

Properties. *Hirsutic Acid C.* Large colorless prisms, odorless, almost tasteless, no water or alcohol of crystallization; crystals strongly pyroelectric; m.p. 179.5° C (uncorr.); 1.5 mg/ml soluble in water at room temperature, more soluble in hot water, barium salt freely soluble in water, soluble in most organic solvents except hexane and carbon tetrachloride; $[\alpha]_D^{20°} = +11.9°$ (free acid in absolute alcohol); no ultraviolet absorption spectrum.

Reactions. *Hirsutic Acid C.* Titrates as a monobasic acid, pK = 4.6; negative Fehling, ammoniacal silver nitrate, 2,4-dinitrophenylhydrazine, acidified KI; decolorizes potassium permanganate, white precipitate with bromine water, faint brown color with ferric chloride; no methoxyl, hydroxyl groups; positive nitro-chromic reaction; ether solution with diazomethane gives orthorhombic plates or needles.

Hirsutic Acid N. Described as a transparent, colorless glaze; almost insoluble in cold water, slightly soluble in hot water, readily soluble in alkali, most organic solvents except hexane and carbon tetrachloride; titrates as a monobasic acid; stable indefinitely as free acid solid or ether solution, less stable in aqueous solution particularly at alkaline pH.

Hirsutic Acid A. Described as a colorless brittle glaze; solubility and analytical reactions like Hirsutic Acid C and N.

BACTERIOLOGY

Assay. Cylinder plate method with *Staph. aureus.*

Spectrum in vitro. *Hirsutic Acid N.* Inhibition dilutions against *Staph. aureus* and *Strep. pyogenes* 1:10,000; against *C. diphtheriae* and *N. meningitidis* 1:40,000; activity in order of decreasing sensitivity, *M. lysodeik-*

ticus, B. anthracis, Sar. lutea, Staph. aureus, V. comma, B. subtilis; no activity against *S. typhi, E. coli, A. aerogenes, K. pneumoniae, Pr. vulgaris, Ps. aeruginosa, Ser. marcescens.*

Hirsutic Acid A. Inhibition dilution against *Staph. aureus* 1:8,000; less active against *Strep. pyogenes;* no activity against *E. coli, S. enteritidis.*

Factors affecting activity. Activity of Hirsutic Acid A not affected by ox serum and peptone.

Mode of action. No visible lysis of *Staph. aureus.*

PHARMACOLOGY

Toxicity. *Hirsutic Acid C.* Intravenous, non-toxic to mice up to 31 mg of sodium salt. *Hirsutic Acid N.* Intravenous MLD 1–2 mg; oral 20 mg, tolerated. *Hirsutic Acid A.* Intravenous MLD 15–20 mg. Hirsutic Acid N and A at 1:4,000 killed human leucocytes.

Physiology. *Hirsutic Acid N.* Increased blood pressure, caused irreversible slowing of isolated heart, no effect on respiration, in cat; caused cessation of uterine contractions of guinea pig. *Hirsutic Acid A.* Had no effect on blood pressure, heart rate, respiration rate.

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HUMULON See LUPULON

HYDROXYASPERGILLIC ACID See ASPERGILLIC ACID

INOLOMIN

First described by Fragner in 1949; derived from *Inoloma traganum.*

PRODUCTION

Isolation. Fresh fungi crushed with water, mash centrifuged and liquid pressed through paper filter, yielding a pale violet colloidal solution; concentrated by evaporation at pH 5–7 at 100° C.

Purification. Filtrate saturated with ammonium sulfate, the precipitate discarded, the clear yellowish filtrate mixed with animal charcoal and filtered; filtrate mixed with "carborafine," filtered, moist "carborafine" extracted with concentrated ethanol, the extract filtered and evaporated in vacuo.

CHEMISTRY

Properties. Yellow glossy substance, very hygroscopic, easily soluble in water and in pH 7 phosphate buffer; becomes inactive and turns brown on autoclaving; stable at room temperature; activity unchanged by Seitz filtration.

BACTERIOLOGY

Crude inolomin inhibited growth of a *Micrococcus* sp.

PHARMACOLOGY

Toxicity. Crude inolomin, 1.0 ml of 10% solution intravenously, was nontoxic to mice.

BIBLIOGRAPHY

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IODININ

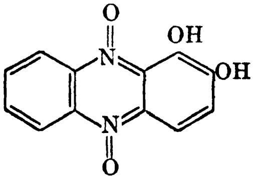
First isolated by Clemo and McIlwain in 1938[1]; named and antibiotic activity described by McIlwain in 1941[2]; derived from *Chromobacterium iodinum*.

PRODUCTION

Fermentation. Surface growth on media containing soluble nitrogenous substances, such as peptone or meat extract, and with citrates and beerwort; no iodinin formation with sugars and most amino acids; incubated several days at 28° C with additional incubation at 15° C.[1]

CHEMISTRY

Constitution. N,N′-dioxide of a dihydroxyphenazine with probable structure[1, 2]:



Properties. Soluble in benzene, toluene, xylene, chloroform, carbon disulfide, ethylacetate; slightly soluble in hot alcohol; insoluble in water, cold alcohol, aqueous alcohol, acetic acid, petroleum ether, amyl alcohol, ether; pK about 12.5; spectrum, general absorption from 4,700–5,700 mμ in benzene.[1, 2]

Reactions. Blue-green, water-soluble salt with alkali, decomposed by heat to a gray colloidal substance, reformed by acidification and addition of benzene; stable with concentrated HCl, formaldehyde, acetic acid plus nitrite, dilute HCl plus zinc, hydrogen peroxide and potassium persulfate; unstable in alkali.[1,2]

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (γ/ml)[3,4]: *Staph. aureus* 8–43, *St. hemolyticus* 0.4–1.7, *Eb. typhi* > 43–85, *Pr. vulgaris* > 85–170, *E. coli* > 85.

Factors affecting activity. Antagonism of activity by anthraquinone and naphthaquinone derivatives.[3,4]

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IRPEXIN

First described by Robbins, Kavanagh and Hervey in 1945; derived from a *Basidiomycetes* sp.

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JAVANICIN

First observed by Wilkins and Harris in 1942[1]; described and named by Arnstein, Cook and Lacey in 1946[3,4]; derived from *Fusarium javanicum;* found in culture liquor in admixture with OXYJAVANICIN.[4]

PRODUCTION

Fermentation. Surface growth on medium containing g/l sodium nitrate 2.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, ferrous sulfate 0.01, K_2HPO_4 1.0, "Tryptone" 5.0, glucose 40, pH 6.8–7.0; incubated 8–12 days at 25° C, final pH 3.0; yield 100 *Staph. aureus* units.[2,3,4,5]

Isolation. (A) Culture filtrate extracted with peroxide-free ether; the ether extract shaken with saturated aqueous sodium bicarbonate to remove impurities, then extracted with aqueous sodium carbonate; the aqueous extract adjusted to pH 7 and reextracted with ether; the ether extract dried and concentrated; yield 46 mg/l.[5] OXYJAVANICIN is also extracted.[4]

(B) Culture filtrate extracted with ether-benzene, the extract shaken with saturated aqueous sodium bicarbonate and with water, then extracted with dilute NaOH; the deep violet-purple aqueous layer separated, acidified with 10% phosphoric acid and extracted into benzene; the benzene layer washed with water, dried and evaporated to dryness in vacuo; yield 23 mg/l.[5] OXYJAVANICIN is also extracted.[4]

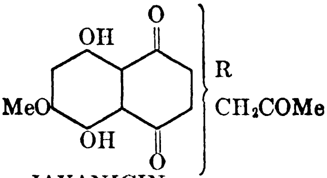
(C) Mycelium separated, dried and powdered; extracted with light petroleum, the extract concentrated; yield 0.1% of dry mycelium.[5]

(D) Mycelium ground and extracted with acetone.[4]

Purification. Crude javanicin extracted with hot acetone-alcohol and crystallized; recrystallized from ethanol.[5]

CHEMISTRY

Constitution. Proposed structure[3, 4]:



JAVANICIN
$C_{15}H_{14}O_6$
R = Me

Javanicin, by analysis, C 62.1, 62.9, H 4.90, 5.11, OMe 10.4, side-chain Me (Kuhn-Roth) 9.2, active H 0.98; calculated C 62.1, H 4.86, 1 OMe 10.7, 2 Me 10.3, 3 H 1.03.[5]

Properties. Red laths with coppery luster; m.p. 207.5–208° C (dec.); very weakly acid.[3, 4, 5]

Reactions. Thermo-, acid- and alkali-stable; stable in solvents; deep violet color with 10% NaOH, purple with ammonia or sodium carbonate; no color with sodium bicarbonate; violet color with lead acetate in methanol; decolorized by bromine water or aqueous sodium bisulfite.[3, 4, 5]

Derivatives. Antibiotically active anhydro- and monoacetyl-derivatives; other derivatives.[3, 4, 5]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus*.[4, 5]

Spectrum in vivo. Inhibition dilution (× 1,000)[3, 4]:

Staph. aureus	200–400	*Ps. aeruginosa*	10
Myco. phlei	100–400	*Ps. fluorescens*	10
B. subtilis	200	*B. tumefaciens*	10
St. pyogenes	40	*Myco. tuberc. hum.*	50–100
B. fasciens	100–200	*St. hemolyticus*	100
E. coli	10	*Cl. welchii*	100

Anti-fungal activity is slight.[4]

OXYJAVANICIN inhibited *Staph. aureus* at 1:200,000.[4]

Factors affecting activity. Not influenced by serum; decomposed by blood yielding brown color (probably methemoglobin).[3,4]

Spectrum in vivo. Javanicin had no marked effect when administered to guinea pigs experimentally infected with *Myco. tuberculosis*.[4]

PHARMACOLOGY

Toxicity. In mice, intraperitoneally, 10 mg was tolerated.[3]

MISCELLANEOUS

Javanicin inhibited germination of seeds and root growth in tomato, lettuce and turnip at 1:20,000.[4]

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KOJIC ACID

First isolated by Saito in 1907[1]; antibiotic activity first observed by Yabuta in 1912[2,3]; derived from *Aspergillus oryzae*, *A. glaucus*, *A. flavus*, *A. gymnosardae*, *A. awamori*, *A. candidus*, *A. clavatus*, *A. fumigatus*, *A. giganteus*, *A. albus*, *A. nidulans*, *A. parasiticus*, *A. tamarii*, *Penicillium daleae* and several species of *Acetobacter*,[4] from *A. effusus*,[4,5,6] from *A. lutescens*,[7] from *A. luteo-virescens*.[8]

PRODUCTION

Fermentation. Surface growth (A) on culture media containing: as carbon sources, glucose or xylose (for best yields), starches, dextrins, disaccharides, monosaccharides, pentoses, sorbitol, dulcitol, *i*-adonitol, inulin, inositol, glycerol, glycero-beta-phosphate, dihydroxyacetone, gluconic acid, and tartaric acid, in concentrations from 5–33%; as nitrogen sources, ammonium nitrate; with inorganic salts, magnesium sulfate and potassium phosphate; at initial pH 2–5. Yields increased by cobaltamines, ethylene chlorohydrin, decreased by oxalic, citric, formic, hydrochloric and nitric acids. Incubated at 29–35° C for 9–20 days; yield 50–60% from glucose by *A. flavus.*[4]

(B) With *A. effusus* on medium containing g/l maltose 40, peptone 10, malt extract 26 ml; incubated at 24° C for 12–14 days; yield 8–9 g/l.[6]

(C) With *A. luteo-virescens* on Raulin-Thom medium with glucose 400 g/l incubated 8–10 days at 28° C.[8]

(D) With *A. lutescens* on 5% malt extract with separately sterilized calcium carbonate incubated at 24° C for 9 days.[7]

Isolation. Culture filtrate cooled for 2 days at 0° C, crystalline kojic acid removed; yield 50 g/l with 20–50 g/l remaining in solution.[8]

Purification. Recrystallization from hot water or by sublimation in vacuo [8]; recrystallization from acetone.[7] Large-scale production.[9]

CHEMISTRY

Constitution. 5-hydroxy-2-hydroxymethyl-γ-pyrone.

Properties. Colorless crystals, m.p. 152° C; red color with ferric chloride; reduces Fehling.[4, 8]

BACTERIOLOGY

Assay. Cylinder-plate with *Staph. aureus* [6]; colorimetric determination with ferric chloride; isolation of copper salt.[8]

Spectrum in vitro. Inhibition of growth of a large number of bacterial species was largely nonspecific, all being inhibited over a range of dilutions of 1:500–4,000,[8] 1:400–6,400 [6]; some evidence for increased activity against gram-negative forms [10]; feeble antiseptic activity against bacteria, yeasts and fungi.[2, 3, 11, 14]

Leptospira canicola was inhibited at 1:1,000,000, *L. icterohemorrhagiae* at 1:100,000.[8]

Factors affecting activity. No effect on activity by increased concentration of inoculum or by incubation with 50% serum.[6]

Spectrum in vivo. Kojic acid did not protect mice against poliomyelitis and St. Louis encephalitis.[12]

PHARMACOLOGY

Toxicity. In dogs, rabbits and rats, an intravenous dose was toxic at 150 mg/kg, lethal at 1 g/kg. In mice, subcutaneous, intraperitoneal and intravenous administration of equivalent doses showed similar toxicity [13]; intraperitoneally, an MLD of 30–40 mg was obtained [8]; orally, 80 mg was lethal.[13]

Human leucocytes were killed at 1:100.[6]

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LACTAROVIOLIN

First described by Willstaedt and Zetterberg in 1946; derived from *Lactarius deliciosus*.

A pigment; $C_{15}H_{14}O$; inhibition of *M. tuberculosis* at 0.31 mM/l.

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LATERITIIN GROUP

First described by Cook, Cox, Farmer and Lacey in 1947 [1] and include: LATERITIIN I from ***Fusarium lateritium***, LATERITIIN II from *Fusa-*

rium sp. (probably a strain of *F. latericium*), AVENACEIN from *F. avenaceum*, FRUCTIGENIN from *F. fructigenum* and SAMBUCININ from *F. sambucinum*.

Lateritiin I is stated to be identical with ENNIATIN A.[1]

CHEMISTRY

Constitution.

	Empirical formula by analysis	M.p. (C°)
Lateritiin I	$C_{26}H_{46}O_7N_2$	121–122
Lateritiin II	$C_{26}H_{46}O_7N_2$	125
Avenacein	$C_{25}H_{44}O_7N_2$	139
Fructigenin	$C_{26}H_{44-46}O_7N_2$	129
Sambucinin	$C_{24}H_{42}O_7N_2$	85–86

Properties. Crystalline, colorless, optically active, neutral compounds; slightly soluble in water, very soluble in organic solvents; heat- and acid-stable, alkali-unstable; on degradation with HCl or HBr yields an optically active crystalline nitrogen base and an optically active alpha-hydroxyisovaleric acid corresponding to *d*(−)valine [1]; from latericiin I, *d*(−)alpha-hydroxyisovaleric acid and *l*(+)N-methylvaline.[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 10,000)[1]:

	Myco. phlei I	*Myco. phlei* II	*S. aureus*	*B. subtilis*	*S. pyogenes*
Lateritiin I	20	32	5	8	4
Lateritiin II	16	32	4	3	2.5
Avenacein	16	16	1	0.5	1
Fructigenin	20	30	2	1	5
Sambucinin	20	4	4	1	2

Lateritiin I inhibited 3 strains of *Myco. tuberculosis* at 1:160,000–640,000.[1]

PHARMACOLOGY

Low toxicity intraperitoneally.[1]

BIBLIOGRAPHY

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LATEROSPORIN

First described by Barnes in 1949; derived from *Bacillus laterosporus;* composed of LATEROSPORIN A and B.

PRODUCTION

Fermentation. Surface growth on medium containing g/l glucose 5, yeast extract 5, KH_2PO_4 0.5, K_2HPO_4 0.5, $(NH_4)_2SO_4$ 5, pH 6.6; incubated at 37° C for 3–4 days, final pH 5.8–6.0; yield 1.0 unit/ml.

Isolation. Culture liquor centrifuged at pH 2.0, the supernatant adjusted to pH 6.5–7.0 and the activity adsorbed on activated carbon; activity eluted with acid aqueous butanol, the butanol separated, ether and water added, the aqueous phase separated.

Purification. Crude laterosporin precipitated with picric acid and extracted with acid ethanol; laterosporin A soluble in acid ethanol, precipitated from dry ether; laterosporin B insoluble in acid ethanol, washed with dry ether; total yield 25–30% with A and B proportions as 1:1.5.

CHEMISTRY

Constitution. Peptide structure.

Properties. Soluble in water at pH 2.0, decreasing in solubility up to pH 9.0; insoluble in ether, chloroform and amyl acetate; dialyzes through cellophane; laterosporin A soluble, laterosporin B insoluble in absolute ethanol.

BACTERIOLOGY

Assay. Cylinder plate with *Myco. phlei*, *C. xerosis* and *Staph. aureus;* arbitrary standard 10 units/mg; laterosporin A 10 units/mg, laterosporin B 12.6 units/mg.

Spectrum in vitro. Laterosporin A or B, inhibition concentration (γ/ml):

Staph. aureus	0.01	*Myco. smegmatis*	10.0
St. pyogenes	0.03–0.12	*Myco. tuberculosis*	1.0
C. diph. gravis	0.12	*S. typhi*	0.6
C. xerosis	0.003–0.005	*S. enteriditis*	1.2–2.5
Myco. phlei	0.004–0.0008	*E. coli*	0.6
Ps. aeruginosa	5.0–10.0		

Factors affecting activity. Activity lowered 60% by horse serum.

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LATEROSPORIN A *See* LATEROSPORIN

LATEROSPORIN B *See* LATEROSPORIN

LAVENDULIN

First described by Kelner, Kocholaty, Junowicz-Kocholaty and Morton in 1946[1]; derived from *Actinomyces* sp., variant of *A. lavendulae.*

PRODUCTION

Fermentation. (A) Surface growth on medium containing g/l peptone 5, beef extract 3, glucose 10, molasses 20, agar 2.5, pH 6; incubated at 28° C for 6 days, final pH 5.6; yield 200–400 *E. coli* units. (B) Submerged growth in shaker flasks on medium g/l peptone 5, glucose 10, molasses 20; incubated 4 days, final pH 7.4; yield 1,000 *E. coli* units.[2]

Isolation. Culture liquor adjusted to pH 7, "Decalso" cation exchanger added and stirred, the supernatant liquid decanted; the sediment washed with water and acetone, dried at 28–30° C, eluted with saturated sodium chloride, eluate evaporated to dryness in vacuo; the residue extracted with boiling absolute methanol; the methanol extract refrigerated, a gummy precipitate separated and discarded; ether added, precipitating crude lavendulin; dried in vacuo to a light-green hygroscopic powder.[3]

Purification. Crude lavendulin dissolved in 85% methanol, passed through an alumina column, aqueous sodium helianthate added to the percolate and the mixture refrigerated, precipitating a crystalline addition compound; recrystallized from aqueous methanol; hydrochloride purified by chromatography.[3]

CHEMISTRY

Constitution. *Of helianthate addition compound.* Proposed $C_{49}H_{63}O_{18}N_{13}S_3$ by analysis, C 51.16, H 5.99, N 17.32, S 9.17; less likely $C_{33}H_{42}O_{12}N_8S_2$, $C_{16}H_{20}O_6N_4S$.

Properties. *Of helianthate addition compound.* Orange needles or irregular triangular blades recrystallized from aqueous methanol, m.p. 212–220° C (corr., dec.). *Of free base.* Positive biuret, Fehling and potassium permanganate; negative Sakaguchi and Molisch; dialyzes through cellophane; thermostable in neutral solution; stable when adsorbed on "Decalso," in sodium chloride eluate and methanol solution.[3]

BACTERIOLOGY

Assay. Serial dilution by streak test with *E. coli;* crude lavendulin 0.5–1 γ/unit, helianthate 0.3–0.5 γ/unit.[3]

Spectrum in vitro. Inhibition concentration (units/ml)[2]:

E. coli	1.0	*Diplo. pneumoniae*	128
Aero. aerogenes	0.5	*E. typhi*	0.015–1.0
Alk. fecalis	4	*E. communior*	1.0
B. anthracis	4–8	*G. tetragena*	0.015
B. cereus	64	*K. pneumoniae*	0.03

B. mesentericus	0.5	*Micro. auranticus*	0.000007
B. mycoides	0.06–32	*Myco. smegmatis*	1.0
B. subtilis	0.03–6	*Myco. tuberc. bov.*	6
C. diphtheriae	4	*Neis. catarrhalis*	0.06
C. xerose	0.007	*Pr. vulgaris*	2
Br. abortus	16	*Ps. aeruginosa*	128
Br. melitensis	8	*Sarcina lutea*	0.5
Br. suis	32	*Ser. marcescens*	4
Ch. violaceum	0.025	*Staph. aureus*	0.015
V. comma	0.25	*Strep. pyogenes*	128
S. enteriditis	0.25	*Strep. alpha*	>128
S. paratyphi	0.06	*Strep. gamma*	128
S. schottmuelleri	0.125	*Shig. dysenteriae*	2
Trich. interdigitale	16	*Shig. paradysenteriae*	2

Factors affecting activity. In 0.8% sodium chloride, activity is reduced to 1/128; no further effect by addition of 10% horse blood.[2]

Spectrum in vivo. In mice intraperitoneally, 25 γ of lavendulin (0.078 γ per *E. coli* dilution unit) gave complete protection against experimental infection with *K. pneumoniae*.[4]

PHARMACOLOGY

In mice, intraperitoneally, the LD 100 was 0.5 mg.[4]

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LECANORIN A

Described by Roques in 1948[1]; derived from *Lecanora esculenta*. (LECANORIC ACID has been isolated from lichens of the genera *Lecanora*, *Roccella* and *Parmelia*).[2]

The aqueous extract inhibited the germination of the seeds of *Lepidium sativa*.[1]

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LICHENIFORMIN

First described by Callow and Hart in 1946[1]; derived from *Bacillus licheniformis.*

PRODUCTION

Fermentation. Surface culture on medium containing K_2HPO_4 1.0 g, sodium citrate 0.5 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, sodium lactate (50%) 10 ml, ammonium lactate (50%) 10 ml, $MnSO_4 \cdot 4H_2O$ 1.0 ml of 0.3% solution, iron ammonium citrate 1.0 ml of 1.7% solution, distilled water 1.0 liter, HCl to pH 7.0; incubated at 37° C for 6 days, final pH 8.8–9.6; yields 400–600 *M. phlei* units/ml.[2,4]

Isolation. Fermented liquor acidified with HCl to pH 2.5, boiled for 20 minutes or autoclaved for 10 minutes, centrifuged, the clarified liquor adjusted to pH 5 with NaOH, a slight precipitate removed by centrifuging, the active material adsorbed on activated carbon, the carbon cake washed with water and with denatured alcohol, eluted with acidified *n*-butanol-saturated water; yield about 50% of initial activity in the culture medium.[2,3,4]

Purification. After volume reduction by in vacuo distillation or repetition of carbon adsorption and elution, eluate adjusted to pH 6, poured into excess picric acid solution; the picrate precipitate is decomposed with HCl and the filtrate added to acetone, yielding a gummy precipitate which is dissolved in methanol and reprecipitated from acetone; final product, a white solid precipitate, filtered and dried in vacuo; yield overall 25%, potency 2.5–5 $\times$ 10^3 *M. phlei* units per mg.[4]

CHEMISTRY

Constitution. C 39.6, H 7.6, N 17.3, Cl 19.8 hydrochloride from recrystallized helianthate; C 41.0, H 7.5, N 17.4, Cl 18.4 fraction eluted from acid-washed alumina.

Properties. *Of hydrochloride:* white amorphous powder; soluble in water and methanol; slightly soluble in ethanol; insoluble in dry *n*-butanol, acetone and non-polar organic solvents; stable in dry state, no loss by autoclaving over pH 1–9, aqueous solution lost 75% activity after 6 months at room temperature in light; rapid loss of activity in concentrated acid or alkali;

activity lost by Seitz filtration at pH above 1.5, activity recovered by elution with dilute HCl; slow dialysis through cellulose membrane; $[\alpha]_D = -37°$, $[\alpha]_{5,461} = -44°$ ($c = 1.09\%$) purified by helianthate crystallization; $[\alpha]_D = -37.5°$, $[\alpha]_{5,461} = -47°$ ($c = 0.93\%$), purified by chromatography.

Reactions. Positive biuret, Sakaguchi; negative ninhydrin, Molisch, glucosamine; precipitate with sodium hypochlorite; sulfate obtained as a white powder consisting of glassy particles, helianthate as microscopic red needles, reineckate as crystals but could not be recrystallized, rhodanilate as an oil, picrate, picrolonate, flavianate as oily semi-solid precipitates, rufianate and oxalate as amorphous solids.[4]

BACTERIOLOGY

Assay. Serial dilution with *M. phlei*, incubated at 37° C for 4 days.

Spectrum in vitro. Inhibition dilution [4]:

Myco. phlei	$2.5–5.0 \times 10^6$
Myco. tuberculosis (4 virulent strains)	$1.6–6.4 \times 10^5$
Coryne. renale	4×10^7
Coryne. diphtheriae (6 strains)	$1.25 \times 10^6–10^7$
Coryne. equi	5×10^6
Coryne. pyogenes (3 strains)	low activity
Coryne. ovis	low activity
Staph. pyogenes (5 strains)	$1.25–5 \times 10^6$
Streptococcus A, B, C, O (13 strains)	$8 \times 10^4–1.25 \times 10^6$
Strep. viridans	2×10^4
Past. septica (7 strains)	$<5 \times 10^3–6.4 \times 10^5$
Brucella abortus (3 strains)	3.2×10^5
B. anthracis	2×10^5
Shig. dysenteriae (3 strains)	$4 \times 10^4–1.6 \times 10^5$
Dip. pneumoniae (3 strains)	$2 \times 10^4–1.6 \times 10^5$
E. coli (5 strains)	$10^4–8 \times 10^4$
Ps. pyocyanea	4×10^4
V. cholera	2×10^4
Sal. paratyphi (2 strains)	$10^4–2 \times 10^4$
Actinobacillus lignieresi (2 strains)	$<10^4$
Erysipelothrix rhusiopathiae (2 strains)	5×10^3

Factors affecting activity. Activity unaffected by 50% horse serum, increased with increase in pH.[4]

Spectrum in vivo. Little to no protection against mice infected with *Staph. pyogenes;* partial protection against *Str. pyogenes;* 0.5–3 mg of licheniformin·HCl ($2.5–5 \times 10^3$ units/mg) intraperitoneally in mice infected with 94.5% lethal dose of *B. anthracis* resulted in 16.2 and 32.5% mortality, respectively; suppressive effect on tuberculosis in aerosol-infected mice, particularly in combination with "sulphetrone." [4]

PHARMACOLOGY

Toxicity. The LD 50 for licheniformin hydrochloride (2.5–5.0 × 10^3 units/mg) injected into mice were: intravenous 5 mg, intraperitoneal 7.5 mg, subcutaneously 10 mg; 6 mg daily subcutaneously for 5–6 weeks showed kidney damage. Did not hemolyze red blood cells in 0.5% solution of licheniformin hydrochloride (4 × 10^3 unit/mg); inhibited phagocytosis in 1% solution; increased clotting time in mice.[4]

Blood levels. Subcutaneous administration of 375,000 units/kg in mice showed a rapid rise to 125 units/ml in blood at 1 hour, falling off rapidly after 2 hours.[4]

Physiology. At 1–2 mg/kg in cats, licheniformin produced sharp drop in blood pressure due to histamine effect; typical "triple response" in human skin at 10^5 dilutions; no action on isolated guinea-pig ileum.[4]

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LITMOCIDIN

First described by Gause in 1946[1]; derived from *Proactinomyces cyaneus* var. *antibioticus*.

PRODUCTION

Fermentation. Surface growth on medium containing peptone 0.5%, "tryptone" 0.3%, glucose 1%, NaCl 0.5%, $FeSO_4$ 0.001%, agar 2%, incubated 48–72 hours at 28° C, yield 2,000 *Staph. aureus* units/ml.[1]

Isolation. Aqueous extract of agar culture acidified to pH 3.5, activity adsorbed on activated carbon, eluted with acid acetone, acetone extract evaporated to dryness in vacuo, residue dissolved in ethanol; crude litmocidin precipitated by addition of water, centrifuged, dried in vacuo.[2]

Purification. Crude litmocidin dissolved in ethanol, HCl, ether and water added, the ether layer evaporated to dryness, residue dissolved in ethanol, purified litmocidin precipitated by addition of water.[2]

CHEMISTRY

Properties. Activity associated with a pigment; dry powder m.p. 144–146° C (decomp.); red in acid, violet in neutral, blue in alkaline solution; acid form soluble in alcohols, acetone, ether, amyl acetate, slightly soluble in water; alkaline form readily soluble in water; blue sediment with lead acetate; decolorized by zinc dust, color restored by exposure to air; decolorized by bisulfite; stable in acid solution, unstable in alkaline; strong alkali destroys activity but color is retained, heating with 20% HCl affects color but does not reduce activity.[2]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* purified litmocidin 4,000 units/mg.[1]

Spectrum in vitro. Inhibition dilution (× 1,000)[1]:

Staph. aureus (4 strains)	2,000–4,000
Strep. hemolyticus (7 strains)	400–2,000
Strep. viridans	500
Vibrio comma (7 strains)	500–2,000
Shig. dysenteriae	10
Shig. paradysenteriae	10
E. typhi	1
S. paratyphi	1
S. schottmuelleri	1
E. coli	1
Myco. tuberculosis	Strong inhibition

Activity is bacteriostatic and bactericidal, unaffected by 10–30% human serum.[1]

Spectrum in vivo. No protection for mice with experimental *Staph. aureus* infection.[1]

PHARMACOLOGY

LD 50 for mice intraperitoneally was approximately 4,000–5,000 units.[1]

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LUPULON

The antibacterial properties of extracts of commercial hops, the dried cones of the hop vine, *Humulus lupulus,* have been known for many years. Furthermore, it has long been recognized in a general way that the compounds, lupulon and HUMULON, found in hops, are antibacterial agents;

their isolation and chemical properties have been studied in detail.[1] However not until very recently have the antibiotic properties of these two compounds been more precisely established, beginning with the work of Walker and Parker and of Shimwell in 1937.[2, 3]

PRODUCTION

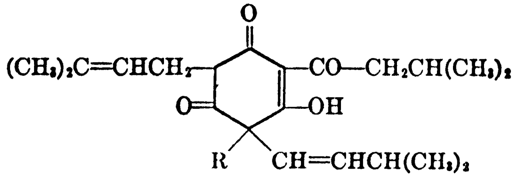
Isolation. *Lupulon.* (A) Ground hops extracted with absolute methanol, the methanol extract diluted with aqueous 2% sodium chloride and extracted with petroleum ether; the petroleum ether extract evaporated to a syrup, cooled overnight and lupulon crystals removed.[4] (B) Hops ground with an equal weight of "Dry Ice" and extracted with petroleum ether; the petroleum ether extract evaporated in vacuo to a syrup and allowed to crystallize at −15° C.[5]

Humulon. (A) Mother liquor from lupulon crystals dissolved in absolute methanol, lead acetate added, the precipitate removed and suspended in ether; lead removed with hydrogen sulfide, the filtrate evaporated to dryness, the residue dissolved in methanol, reprecipitated with lead acetate and recovered as previously, the final residue allowed to crystallize in the cold; yield 2.0 g per 1,025 g hops.[4] (B) Precipitated from crude petroleum ether extract of hops or from mother liquor of lupulon crystals with *o*-phenylenediamine; free humulon by acidification and extraction with an organic solvent such as ethyl ether; yield 80%.[5]

Purification. *Lupulon.* (A) Recrystallized from 70% alcohol; yield 1.34 g per 1,025 g hops.[4] (B) Dissolved in warm petroleum ether, recrystallized by chilling in "Dry Ice"; crystals dissolved in methanol, filtered and recrystallized from 90% methanol; yield 1.5–3.0%.[5]

CHEMISTRY

Constitution[1]:



Lupulon: R = —CH=CHCH(CH$_3$) 2, $C_{26}H_{38}O_4$; M.W. 414.3.[1]

Humulon: R = —OH, $C_{21}H_{30}O_5$; M.W. 362.3.[1]

Properties. *Lupulon.* Fine white crystals; m.p. 90.5–92° C, 92° C; turns yellow and amorphous in a few days; optically inactive. *Humulon.* Yellow crystals; m.p. 55° C, 65–66.5° C; $[\alpha]_D^{20°} = -232$ (benzene).[1]

Fairly soluble in methanol, ethanol, petroleum ether, hexane, iso-octane; slightly soluble in neutral or acid aqueous solution; readily soluble as sodium salt.[5]

Reactions. *Lupulon.* Moderately stable in acid and alkali; oxidized on exposure to air, oxidation inhibited by ascorbic acid even at autoclave temperature.[5, 9] *Humulon.* Relatively stable in acid; lead salt stable but not *o*-phenylenediamine salt; oxidized on exposure to air; unstable in alkaline solution; oxidation inhibited by ascorbic acid even at autoclave temperature.[5, 9]

Yellow alkali salts; red-violet color with ferric chloride; decolorized permanganate and bromine water; catalytically hydrogenated.[1]

BACTERIOLOGY

Assay. Agar plaque of test organism; serial dilution with *Sclerotina fructicola* spore germination, 50% germination with 150 γ lupulon per ml [4]; turbidimetric and cylinder-plate.[5]

Spectrum in vitro. Inhibition dilution (× 1,000) [5]:

	Lupulon	Humulon
B. anthracis	300	100
B. cereus v. mycoides	1,000	100
B. subtilis	1,000	50
C. diphtheriae gravis	100	10
D. pneumoniae I	300	20
Micro. lysodeikticus	300	60
Micro. pyogenes v. aureus	500	30
Sarcina lutea	100	30
St. fecalis	500	30
Myco. phlei	300	30
Myco. tuberculosis v. hom.	100	10
Streptomyces coelicolor	50	3
13 gram-negative sp.	<3	<3
4 yeast sp.	<3	<3
7 fungi sp.	<3	<3

Lupulon, 50% inhibition concentration (γ/ml) [4]:

Rhizopus nigricans	40	*Phytophthora citrophthora*	600
Pythium sp.	500	*Fusarium lycopersici*	300
Rhizoctonia solani	150	*Penicillium digitatum*	250
Sclerotium bataticola	80	*Botrytis cinerea*	200
Aspergillus niger	1,600	*Alternaria citri*	900
Aspergillus oryzae	200	*Sclerotinia fructicola*	40

Lupulon inhibited *Staph. aureus* at 1.5 γ/ml, *Myco. phlei* at 50 γ/ml, *Myco. tuberculosis* hominis at 25 γ/ml [8]; inhibited *Myco. tuberculosis* at 1:40,000–200,000 [6, 7]; much lower activity with humulon.[6]

Factors affecting activity. In serum, no reduction in activity against *Myco. tuberculosis*, 30% reduction against *Myco. phiei*, 90% reduction against *Staph. aureus;* slight reduction of activity against *Staph. aureus* with increased inoculum; increased activity at low pH and in the presence of sodium chloride [8]; no synergism with other antitubercular agents.[6]

Spectrum in vivo. In mice, experimentally infected with *Myco. tuberculosis*, treated with lupulon intramuscularly 60 mg/kg/day with 1.5% solution in cottonseed oil for 30 days, 75% reduction of mycobacteria in organs; similar result by oral administration of 150 mg/kg twice a day with 3% lupulon in 6% aqueous gum acacia for 30 days [6]; no protection against experimental infections with *St. pyogenes*.[7]

PHARMACOLOGY

Toxicity. In mice, intramuscularly, LD 50 = 600 mg/kg of 1.5% solution of lupulon in cottonseed oil; 60 mg/kg/day for 4 weeks tolerated; orally, LD 50 = 1,500 mg/kg of 5% in 6% aqueous gum acacia; renal tubule pathology with intramuscular administration at therapeutic levels.[6]

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LYCOPERSICIN *See* TOMATINE

MARASMIC ACID

First observed by Wilkins in 1945 [1]; described and isolated by Kavanagh, Hervey and Robbins [2]; derived from *Marasmius conigenus*. Two other antibacterial substances may also be produced by *M. conigenus*.[2]

PRODUCTION

Fermentation. Surface growth on medium containing corn steep liquor and beechwood shavings; incubated about 4 weeks; yield 256–512 *Staph. aureus* units/ml; additional yields by draining and reflooding mycelium with fresh medium.[2]

Isolation. Culture liquor acidified to pH 2 with HCl, extracted with chloroform; chloroform extract concentrated in vacuo, washed with pH 5.8 phosphate buffer, and evaporated to dryness in vacuo; the residue dissolved in ether, extracted with 2% sodium bicarbonate solution; the bicarbonate extract acidified, reextracted with ether; the ether extract evaporated slowly, the crystalline residue washed with ether.[2]

Purification. Recrystallization from ether, dilute aqueous alcohol or acetone, water or ether-hexane; maximum yield 100 mg/l.[2]

CHEMISTRY

Constitution. Proposed $C_{16}H_{26}O_4$, C 69.6, H 7.25; found C 68.21, H 7.17; M.W. 276; equivalent weight 276 [2]; unsaturated, with a carbonyl and probably a carboxyl group.[2]

Properties. Long white needles; m.p. 165° C (uncorr.) in open capillary; 174–175° C (corr.) in sealed capillary in vacuo [1]; soluble in acetone, ethanol, ether, chloroform, water (1.1 mg/ml at 25° C); insoluble in hexane; $[\alpha]_D^{25}$ + 176° (1.4% in acetone); ultraviolet absorption spectrum in alcohol, 240–242 and 314–318 mμ with ϵ at 10.4 and 45.6 respectively; in phosphate buffer at pH 1.45–3.52, 5.4, 8.8 and 11.0, 245, 246, 248 and 260 mμ with ϵ at 11.4, 10.4, 8.4 and 3.9, respectively; pK in 30% alcohol 5.8 (monobasic acid).[2]

Reactions. Reduced Fehling, ammoniacal silver nitrate; reduced permanganate in sodium carbonate solution but not in acid solution; increased reducing properties at high pH; no reaction with hydroxylamine hydrochloride, with bromine in carbon tetrachloride, with alcoholic ferric chloride; red color with alkaline nitroprusside, changing to pale gray-green on acidification; orange-colored 2,4-dinitrophenyl hydrazone m.p. 136–138° C (corr.); one mole of cyanide added for each equivalent of acid by Kiliani reaction in 0.1*N* acetic acid.[2]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* antiluminescent activity with *Ph. fischeri.*[2]

Spectrum in vitro. Inhibition concentration (mg/ml) [2]:

B. mycoides	1	*Myco. smegmatis*	32
B. subtilis	1	*Photo. fischeri*	0.016
E. coli	64	*Ps. aeruginosa*	250
K. pneumoniae	128	*Staph. aureus*	2

A. niger	32	*P. notatum*	32
Chaet. globosum	64	*Phyco. blakesleanus*	16
Gliomastix convoluta	250	*Sacch. cerevisiae*	32
Memnonialla echinata	64	*Stemphylium consortiale*	128
Myrothecium verrucaria	64	*Trich. mentagrophytes*	4

Factors affecting activity. Activity against *Staph. aureus* decreased by human blood.[2]

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MICROCOCCIN

First described by Su in 1948; derived from a *Micrococcus* sp. resembling *M. variens*.

PRODUCTION

Fermentation. Surface growth on medium containing g/l meat extract 10, peptone 10, NaCl 5, pH 7.4; incubated at 37° C for 3 days, final pH 8.4–8.6.

Isolation. (A) Whole culture liquor acidified with HCl to pH 2.0, the precipitate separated and extracted with alcohol, the alcohol removed by distillation; the aqueous residue extracted with chloroform and crude micrococcin precipitated from the chloroform extract with ether. (B) Activity of the culture filtrate adsorbed on activated carbon, the carbon washed with alcohol; micrococcin eluted with pyridine, the eluate evaporated *in vacuo*, the residue dissolved in alcohol and precipitated with ether.

Purification. Crude micrococcin dissolved in chloroform, chromatographed over alumina, eluted with alcohol and the eluate evaporated to dryness.

CHEMISTRY

Constitution. Contains N and S, no halogens.

Properties. An amorphous cream-colored powder; m.p. 220–223° C; soluble in alcohol, acetone, chloroform, propylene glycol, glacial acetic acid, pyridine; slightly soluble in water; insoluble in ether, benzene, amyl acetate, glycerin, vegetable oils; dialyzes through cellophane; $[\alpha]_D^{20} = +50$–$75°$ (alcohol); in ultraviolet, blue fluorescence and maximum at 345 mμ, $E_{345\,m\mu}^{1\,cm} = 0.30$ (0.02 mg/ml in alcohol).

Reactions. Heat-stable; alkali-labile; negative ninhydrin; unaffected by trypsin, pepsin and cysteine.

BACTERIOLOGY

Assay. Cylinder plate; serial dilution on agar slant with *B. subtilis.*

Spectrum in vitro. Inhibition concentration (γ/ml):

Staphylococcus	0.03–0.10	*C. diph. mitis*	0.10
Staph. albus	0.10	*C. diph. gravis*	0.10
Streptococcus	0.10	*C. xerosis*	0.016
St. fecalis	0.025	*Cl. sporogenes*	6.25
B. subtilis	0.016	*Cl. welchii*	3.13
B. anthracis	0.10	*Myco. tuberculosis*	6.25
Streptomyces sp.	0.10	*V. comma*	0.10
E. coli, Ps. aeruginosa, S. typhi, Pr. vulgaris			>1,000

Factors affecting activity. Unaffected by serum, blood.

Mode of action. Bacteriostatic on growing cells.

Resistance. Resistant strains with *Staph. aureus;* no cross-resistance with penicillin.

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MICROMONOSPORIN

First described by Waksman, Geiger and Bugie in 1947; derived from *Micromonospora* sp.

PRODUCTION

Fermentation. Submerged growth on "Tryptone"-starch media, 5–7 days' incubation; yield: 1,000–3,000 *B. subtilis* units, 100 *Staph. aureus* units.

Isolation. *Culture filtrate.* Saturated with ammonium sulfate, precipitating a chocolate-brown sediment; precipitate dissolved in 5% NaCl, dialyzed against tap water and freeze-dried; yield 78 mg from 1.5 liters of filtrate.

Mycelium. Extracted with acetone to give a bright orange solution, evaporated to dryness; yield 168 mg from 1.5 liters.

CHEMISTRY

Constitution. Micromonosporin from culture filtrate: 6.7% N, 5.5% NH_4 nitrogen. Considered a highly pigmented unstable protein associated with a carbohydrate.

Properties. Non-dialyzable through collodion, adsorbed on activated carbon and eluted by water, buffers or common organic solvents; inactivated at pH below 3.0 or above 9.0; 66% loss of activity by 1 hr. at 100° C; not digested by pepsin or trypsin; positive Molisch test, negative phloroglucinol, orcinol and naphthoresorcinol.

Micromonosporin from culture mycelium: considered a quinone; no color change with alcoholic ferric chloride; orange to red color by dilute KOH; color bleached to yellow by sodium hydrosulfite, iodine from HI; Wurster reagent not affected.

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (units/mg × 1,000):

	From filtrate	From mycelium
Staph. aureus	30	200
B. mycoides	80	200
B. subtilis	800	20,000
Sar. lutea	300	6,000

No antibiotic activity against gram-negative bacteria.

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Waksman, S. A., Geiger, W. B., and Bugie, E., "Micromonosporin, an antibiotic substance from a little-known group of microorganisms," *J. Bact.*, **53**, 355 (1947).

MUSARIN

First described by Thaysen and Butlin in 1945 [1, 2]; named by Arnstein, Cook and Lacey [3] from the banana plant, *Musa sapientum*, derived from "Meredith's actinomycete." [4, 5] (The naming of this antibiotic represents a departure from customary procedure in that the name is taken from the banana plant, host to the parasitic *Fusarium oxysporum* var. *cubense* or "Panama disease," rather than from the antibiotic-producing actinomycete [3]).

PRODUCTION

Fermentation. *Surface growth.* (A) On medium containing g/l KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, K_2HPO_4 1.0, glucose 10 (optional), with yeast extract, malt extract, peptone or casein; incubated at 25° C for 12 days; yield 8 *F. lateritium* spore units/ml.[3] (B) In flasks with a layer of malt extract agar with added peptone-glucose solution; yield 8 units/ml.[3]

Submerged growth. (A) In shaker flasks incubated for 14 days; yield 2 units/ml. (B) In aerated tank on medium containing g/l glucose 10, NaCl 0.5, K_2HPO_4 0.5, $FeSO_4 \cdot 7H_2O$ 0.025, "Eupepton" 2.5, "Bacto-tryptone" 2.5; incubated at 20–25° C for 4–5 days; yield 32 units/ml.[3]

Isolation. (A) Culture filtrate activity precipitated with ammonium sulfate; precipitate extracted with methanol. (B) Culture filtrate acidified with phosphoric acid to pH 3.5–4.0, the precipitate extracted with neutral phosphate buffer; the buffer extract shaken with *n*-butanol, the *n*-butanol extract evaporated *in vacuo* to dryness; the residue taken up in methanol and precipitated with ether; yield 42%. (C) Varying yield by extraction of mycelium with methanol.[3]

Purification. No further purification by chromatography over alumina.[3]

CHEMISTRY

Constitution. Proposed $C_{35}H_{60}O_{14}N_2$, C 57.35, H 8.2, N 3.8; found C 57.75, H 8.34, N 3.70; equivalent weight ca. 5,000 (titration).[3]

Properties. Light yellow powder; decomposes at 170° C; free acid soluble in aqueous acetone and alcohol, insoluble in water and acetone; sodium or potassium salt soluble in water, methanol, ethanol and butanol, insoluble in ether and acetone; $[\alpha]_D^{20} = +35.1 \pm 1.6$ ($c = 1.21$ methanol at 80 units/mg), $+38.7 \pm 2.7$ ($c = 0.736$ methanol); ultraviolet maxima at 240, 267 mμ, $E^{1\%}_{1\,\mathrm{cm}} = 375{,}200$ (ethanol).[3]

Reactions. An acid; free acid unstable, sodium salt fairly stable in aqueous solution; precipitated by mineral acids, barium chloride, mercuric chloride and copper acetate; negative Molisch, Millon, biuret, sulfuric acid in acetic acid or with iodine, Salkowski, Lieberman, muroxide; weak or no ninhydrin; positive Axenfeld; inactivated product with diazomethane or methanolic·HCl.[3]

BACTERIOLOGY

Assay. With test organism *Fusarium oxysporum* var. *lateritium:* (1) agar-well-plate, (2) serial dilution, (3) inhibition of spore germination, (4) agar-plate dilution, (5) cylinder-plate (small zones due to poor diffusion); purified musarin 100 units/mg.[3]

Spectrum in vitro. With musarin at 80–100 units/mg:

Spore germination inhibition dilution (× 10,000).[3]

F. o. var. *cubense*	10	*Rhizopus stolonifer*	5
F. o. var. *lateritium*	10	*Corticum solani*	80
F. o. var. *culmorum*	10	*Sclerotinia fructigena*	80
F. o. var. *lini*	5	*Aspergillus parasiticus*	5
Botrytis cinerea	40	*Aspergillus niger*	10
Verticillium dahliae	80	*Penicillium notatum*	5
Verticillium albo-atrum	80	*Melanospora destruens*	40
Ceratostomella paradoxa	40	*Alternaria* sp.	40

Growth inhibition dilution (× 10,000)[3]

Staph. aureus	50	*St. pyogenes*	2.5
B. subtilis	10	*Myco. phlei*	10
	E. coli	<0.5	

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(4) Meredith, C. H., "The antagonism of actinomyces to *Fusarium oxysporum cubense*," *Phytopath.*, **33,** 403 (1943).

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MYCELIANAMIDE

First observed by Anslow and Raistrick in 1931 [1]; described by Oxford, Raistrick and Simonart in 1935 [3]; named by Oxford and Raistrick in 1948 [4]; derived from *Penicillium griseofulvum*.

PRODUCTION

Fermentation. Surface growth on medium containing g/l $NaNO_3$ 2–2.5, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, $FeSO_4 \cdot 7H_2O$ 0.02, glucose 50–80 [1, 3]; incubated with shaking at 25° C for 35 days [1] or at 30° C for 65–85 days.[3] Also with ammonium tartrate as nitrogen source.[4]

Isolation. Mycelium removed, pressed or washed with water, dried in vacuo; dried mycelium powdered, extracted with ether; ether extract evaporated to yield white crystals [1]; or dried mycelium extracted with petroleum ether, extract evaporated to dryness, residue extracted with ether, yielding 9.6% crude mycelianamide from dried mycelium.[3]

Purification. Extracted with boiling benzene, cooled, crystallized; recrystallized from ethyl acetate; yield 3.4% of dried mycelium.[3, 4]

CHEMISTRY

Constitution. Proposed $C_{22}H_{28}O_6N_2$, C 65.97, H 7.05, N 7.00; M.W. 400; found C 66.14, H 7.08, N 7.12; M.W. 400.5 (cryoscopic in dioxane); neutralization equivalent 228 (indefinite); no basic or phenolic properties; very weakly acid; no carbonyl group; may be the amide of *o*-mycelyl-N-pyruvyl-beta-ketotyrosine.[4]

Properties. Shiny leaflets; m.p. 170–172° C (dec.); soluble in acetone, dioxane; sparingly soluble in ethanol, chloroform, ether, benzene, glacial acetic acid; soluble in sodium carbonate solution, insoluble in dilute sodium bicarbonate, barium hydroxide or HCl; $[\alpha]^{19}_{5,461} = -217°$, $[\alpha]^{19}_{5,790} = -182°$ ($c = 0.8688$ in chloroform).[4]

Reactions. Thermostable; in cold dilute sodium hydroxide, foams and darkens producing a lavender-like odor; forms insoluble salts, reddish brown ferric, green copper and nickel, yellow silver turning to black; negative Millon, muroxide, biuret, *p*-dimethylaminobenzaldehyde; red-brown color with dilute ferric chloride in ethanol or glacial acetic acid, color discharged by HCl; yellow to brown-red with 70% sulfuric acid; no picrate or methiodide.[4]

BACTERIOLOGY

Spectrum in vitro. At 1:20,000–50,000, inhibited *Staph. aureus*, *Strep. viridans*, *Strep. pyogenes*, *Staph. albus*, *B. anthracis;* no activity against gram-negative bacteria.[4]

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MYCETIN

First described by Fainshmidt and Korieniako in 1944 [1]; derived from *Streptomyces violaceus.*

PRODUCTION

Fermentation. Surface growth on nutrient agar; incubated at 40–50° C for 14–15 days.[1]

Isolation. Dried agar culture extracted with alcohol-benzene (or dichlorethane or chloroform); extract evaporated and residue taken up in alcohol.[1]

CHEMISTRY

Deep violet color in alcohol solution; soluble in alcohol, benzene, chloroform, dichlorethane; slightly soluble in water; insoluble in ether; thermostable.[1]

BACTERIOLOGY

Active against gram-positive bacteria, not against gram-negative; moderately active against corynebacteria and mycobacteria; resistant strains in many susceptible species; antagonized by proteins, pus and in mixed cultures.[2]

BIBLIOGRAPHY

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(2) Krassilnikov, N. A., and Korieniako, A. I., "Mycetin and its bactericidal properties," *Mikrobiologiya* (*U.S.S.R.*), **14**, 80 (1945).

MYCOCIDIN

First described by Gerber and Gross in 1945[1]; derived from a member of the *Aspergillaceae*.

PRODUCTION

Fermentation. Surface growth on modified Czapek-Dox medium, incubated for 14 days.[1]

Isolation. Culture filtrate acidified to pH 1–2, extracted with ether, the ether extract shaken with dilute sodium hydroxide, the aqueous phase neutralized with dilute HCl yielding a highly colored product.[1]

CHEMISTRY

Insoluble in acid; acid precipitate soluble in water, ether, ethyl acetate or alkali.[1]

BACTERIOLOGY

Assay. Serial dilution with *M. tuberculosis* hominis.[2]

Spectrum in vitro. Crude extract at 0.625–1.700 *M. tuberculosis* units/ml inhibited *M. tuberculosis* var. *hominis* in Long's medium or 0.5–0.6 units/ml per mg of *M. tuberculosis*.[2]

Spectrum in vivo. Tubercle bacilli treated with mycocidin prior to injection into guinea-pigs were unable to cause infection.[2]

BIBLIOGRAPHY

(1) Gerber, I. E., and Gross, M., "The inhibition of growth of *Mycobacterium tuberculosis* by a mold product," *Science*, **101**, 616 (1945).

(2) Gerber, I. E., and Gross, M., "Inhibition of growth of *Mycobacterium tuberculosis* by a mold product; the effect on pathogenic human tubercle bacilli," *Science*, **103**, 167 (1946).

MYCOINES

Bactericidal substances allegedly found in *Aspergillus*, *Fusarium*, *Cephalosporium*, *Microsporum*, *Epidermophyton*, *Achorion* and *Actinomycetes*.

Vonkennel, J., Kimmig, J., and Lembke, A., "Mycoines, a new class of therapeutically active substances extracted from fungi," *Klin. Wochenschr.*, 22, 321 (1943).

MYCOMYCIN

First described by Johnson and Burdon in 1947; derived from an actinomycete.

PRODUCTION

Fermentation on "Tryptone"-starch media; extracted with ether or amyl acetate.

BACTERIOLOGY

Assay. Serial dilution with *B. subtilis.*

Spectrum in vitro. Crude extract inhibited *B. subtilis* at 1:7,500 and a virulent *M. tuberculosis* at 1:5,000.

Factors affecting activity. Serum did not greatly lower activity.

PHARMACOLOGY

Toxicity. Crude concentrates were nontoxic to mice. Erythrocytes showed a definite adsorption curve over 24 hours.

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(1) Johnson, E. A., and Burdon, K. L., "Mycomycin: a new antibiotic produced by a moldlike actinomycete active against the bacilli of human tuberculosis," *J. Bact.*, **54**, 281 (1947).

MYCOPHENOLIC ACID

Stated to have been first isolated and crystallized by Gosio in 1896; the first antibiotic to be isolated in crystalline form [1, 8]; named by Alsberg and Black in 1913 [2]; derived from *Penicillium brevi-compactum* and from *P. stoloniferum.*[8]

PRODUCTION

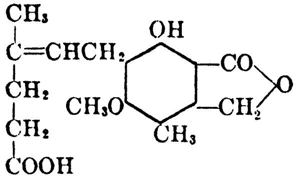
Fermentation. Surface growth on: (A) Medium containing malt extract 2% or on Czapek-Dox with corn steep liquor; incubated at 24° C for 7–14 days [3, 4]. (B) Raulin-Thom, incubated at 24° C for 34 days.[7]

Isolation. (A) Culture liquor acidified to pH 2 with HCl and extracted with amyl acetate; the extract evaporated *in vacuo* to an oil, the residue dissolved in alcohol and precipitated with alcoholic KOH; the precipitate dissolved in water and reprecipitated with acid.[4] (B) Culture filtrate evaporated *in vacuo,* extracted with ether.[7]

Purification. (A) Crystallized from hot water; the sodium salt dissolved in water and treated with activated carbon; precipitated with sulfuric acid and recrystallized from hot water.[4] (B) Crystallized from acetone-chloroform and from boiling water.[7]

CHEMISTRY

Constitution. Proposed structure [9]:



Proposed $C_{17}H_{20}O_6$, C 63.73, H 6.30, OCH_3 9.69, M.W. 320.2; found C 63.85, 63.80, H 6.36, 6.33, OCH_3 9.65, 9.36, M.W. 337, 341.[7, 8]

BACTERIOLOGY

Assay. Cylinder-plate with *Staph. aureus*.[4]

Spectrum in vitro. Inhibition dilution (× 1,000) [4, 5]:

Staph. aureus	1–6.4	*C. diphtheriae*	<4
B. subtilis	20	*St. pyogenes*	<1
C. michiganense	320	*S. typhi*	<1
C. sepedonicum	160	*E. coli*	<1
C. xerosis	4	*Actinomyces scabies*	5–10
Xanthomonas begoniae	10	*Phytophthora erythroseptica*	20
Claviceps purpurea	20	*Rhizoctonia crocorum*	20
Stereum purpureum	80	*Rhizoctonia solani*	5
Verticillium dahliae	20		

Mycophenolic acid did not inhibit *Leuconostoc* sp., *B. tumefaciens*, *B. acoidcae*, *Ps. marginalis*, *Ps. syringae*, *X. campestris*, *X. malcacearum* [5]; inhibited many members of the *Microsporum*, *Endothrix*, *Ectothrix*, *Favus*, *Epidermophyton*, *Endodermophyton* and certain plant-pathogenic fungi at 1:5,000–80,000.[4]

Factors affecting activity. Some reduction of activity by serum.[4]

Mode of action. Mycophenolic acid hindered the synthesis or utilization of a growth intermediate in the metabolism of *Staph. aureus*, causing an increased lag phase.[6]

Resistance. *Staphylococcus aureus* rapidly acquired resistance to mycophenolic acid by serial transfer.[4, 6]

PHARMACOLOGY

Toxicity. In mice, intravenously, 10 mg was lethal; subcutaneously, 10 mg tolerated; oral, 40 mg lethal. No reaction on human skin upon application of ointment containing mycophenolic acid; toxic to leucocytes at 1:200.[4]

Excretion. Trace of activity found in urine; probably inactivated in tissues.[4]

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(9) Birkinshaw, J. H., Bracken, A., and Morgan, E. N., "The constitution of mycophenolic acid," *Biochem. J.*, **42,** xxxlx (1948).

MYCOSUBTILIN

First observed by Michener and Snell in 1947 [1]; isolated and named by Walton and Woodruff in 1949 [2]; derived from *Bacillus subtilis* (SUBTILIN-producing strain); named from its antifungal activity.

PRODUCTION

Fermentation. Submerged growth in shaker flasks on medium containing (A) 20% beet molasses 25%, diammonium phosphate 0.8%, $MnSO_4 \cdot 4H_2O$ 0.005%; (B) g/l sucrose 100, citric acid 11.7, Na_2SO_4 4, yeast extract 5, diammonium phosphate 4.2, KCl 0.76, $MgCl_2 \cdot 6H_2O$ 0.42, $ZnCl_2$ 0.0104, $FeCl_3 \cdot 6H_2O$ 0.0245, $MnCl_2 \cdot 4H_2O$ 0.0181, pH 6.8–6.9 with ammonia; incubated at 25° C for 5 days; yield approximately 160 *Trichophyton* sp. units/ml.[2]

Isolation. Culture liquor adjusted to pH 2.5 with HCl, centrifuged and supernatant discarded; bacterial cell solids extracted with ethanol; ethanol extract added to two volumes of water, precipitating active material; precipitate extracted with ethanol; ethanol extract evaporated to dryness; residue taken up in pyridine, precipitated by addition of ten volumes of water and cooling; yield approximately 30% of crude crystalline mycosubtilin.[3]

Purification. Recrystallization from pyridine and 70% ethanol; potency 700 *Trichophyton* sp. units/mg.[2]

CHEMISTRY

Constitution. By analysis, C 55.31, 55.12, H 7.61, 7.33, N 15.15, 15.18; titration equivalent 1980; by microbiological assay, aspartic acid 45%, tyrosine 5.4%, proline 4.2%.[2]

Properties. White crystals, m.p. 256–257° C (corr.); soluble in pyridine, 70% ethanol, dilute NaOH; practically insoluble in other solvents; ultraviolet spectrum in alcohol-water, 2,770 Å with $\epsilon_{1\,\mathrm{cm}}^{1\%} = 14.6$.[2]

Reactions. Negative with 2,4-dinitrophenylhydrazine, Fehling, ferric chloride; positive with Millon, nitric acid, ninhydrin.[2]

BACTERIOLOGY

Assay. Streak-plate method with *Trichophyton* sp.; inhibition by 0.0015 mg/ml.[2]

Spectrum in vitro. *Bacterial:* Inhibition of *Micrococcus lysodeikticus* by 0.001 mg/ml; no inhibition in 16 bacterial species by 0.016 mg/ml.[2]

Yeasts. Inhibition concentration (γ/ml) [2]:

Torulopsis delbruckii	3.75	*Saccharomyces carlsbergensis*	7.5
Hansenula anomala	3.75	*Mycoderma valida*	7.5
Dipodescus uninuceatus	3.75	*Zygopichia californica*	>20
Torula cremoris	5.0	*Schwanniomyces occidentalis*	>20
Sporobolomyces roseus	5.0	*Debaryomyces gruetzii*	>20
Rhodotorula rubra	ca. 5.0	*Candida guilliermondi*	>20

Fungi. Inhibition concentration (γ/ml) [2]:

Trichophyton sp.	1.5	*Nematospora coryli*	7.5
Ustilago zeae	1.5	*Fusarium moniliforme*	7.5
Sclerotinia fructicola	2.5	*Trich. mentagrophytes*	10.0
Epiderm. inguinale	5.0	*Microsporon lanosum*	10.0
Crypto. neoformans	5.0	*Rhiz. javanicus takela*	>20
Achorion schoenleinii	5.0	*Minor flavus*	>20
Microsporum audouini	5.0	*Trichoderma* sp.	>20
Chaetomium bostrychodes	5.0	*Aspergillus niger*	>20
		Penicillium notatum	7.5

Factors affecting activity. Complete antagonism by 10% horse serum.

PHARMACOLOGY

Toxicity. In mice subcutaneously, 0.25 mg was tolerated, 0.5–1.0 mg was lethal.[2]

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NEBULARINE

First described by Löfgren, Takman and Hedström in 1949; derived from *Agaricus* (*Clitocybe*) *nebularis*.

PRODUCTION

Isolation. Acetone added to press-juice from ground fresh fungi, the precipitate removed and the filtrate evaporated *in vacuo;* the aqueous residue washed with chloroform and with ether, the dissolved organic solvent removed *in vacuo;* the activity of the aqueous residue then adsorbed on activated carbon; the activity eluted with 0.2% 2-octanol in 30% alcohol, the eluate concentrated *in vacuo.*

Purification. The crude concentrate chromatographed over alumina, the middle fractions concentrated *in vacuo* and the chromatography repeated.

CHEMISTRY

Soluble in water and methanol; insoluble in ether and chloroform; neutral reaction; thermostable; precipitates with Reinecke's salt.

BACTERIOLOGY

In a solution containing 1.7% solids, nebularine was active against human, bovine and avian mycobacteria at 1:100,000–1,000,000; not inhibited by serum.

PHARMACOLOGY

Nontoxic to mice subcutaneously at 1:1,000,000.

BIBLIOGRAPHY

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NEOMYCIN

First described by Waksman and Lechevalier in 1949 [1]; derived from *Streptomyces fradiae.*

Neomycin is a generic term for a "neomycin complex" of antibiotics, of which Neomycin A has been isolated.[5]

PRODUCTION

Fermentation. Submerged growth in shaker flasks on medium containing soya peptone 2%, glucose or starch 1%, NaCl 0.5%, meat extract or yeast extract 0.5%, $ZnSO_4 \cdot 7H_2O$ 0.001%; incubated 6 days at 28° C; yield 900 *E. coli* units/ml.[2]

Isolation. Culture filtrate acidified to pH 2.0–2.5, treated with activated carbon (to remove most of another antibiotic with a bacterial spectrum similar to that of neomycin but also demonstrating antifungal activity); filtrate from carbon neutralized; activity adsorbed on "Decalso," eluted with 10% ammonium chloride, activity readsorbed on activated carbon, eluted by 0.05*N* HCl in 50% methanol, neutralized and concentrated in vacuo; residue dissolved in water and freeze-dried or dissolved in methanol and precipitated with acetone.[3]

Purification. Chromatography on alumina or activated carbon column with methanol as solvent and eluant; also precipitation with picric acid followed by conversion to the hydrochloride; by countercurrent distribution.[3,5]

Purification of Neomycin A. Purified neomycin, 500–700 units/mg, treated with sodium *p*-(*p*-hydroxyphenylazo)-benzene-sulfonate, with Methyl Orange and with Orange II to yield crystalline sulfonic acid salts.

Neomycin *p*-(*p*-hydroxyphenylazo)-benzene-sulfonate prepared in 20% aqueous methanol, recrystallized five times from same solvent, regenerated with aqueous hydrochloric acid and butanol, precipitated with acetone.[5]

CHEMISTRY

Properties. A basic compound; soluble in water, slightly soluble in methanol, insoluble in other organic solvents; thermostable in neutral solution and at pH 2.0; negative Sakaguchi.[1,2,3]

Properties of Neomycin A. White amorphous powder; m.p. 250–260° (dec.); $[\alpha]_D^{25} = +83°$ ($c = 1.0$ in water); end absorption in ultraviolet; positive ninhydrin, glucosamine; negative maltol, Sakaguchi.[5]

BACTERIOLOGY

Assay. Agar plate dilution and cylinder plate with *E. coli*[2,3] and *B. subtilis*[5]; purified neomycin 500–700 units/mg[2,3]; Neomycin A 1,700 units/mg; Neomycin A *p*-(*p*-hydroxyphenylazo)-benzene-sulfonate 650 units/mg.[5]

Spectrum in vitro. Inhibition concentration (units/ml)[2]:

A. aerogenes	0.625	*Mall. mallei*	>10.0
B. anthracis	0.156	*Myco. avium*	0.1–0.3
B. mycoides	0.1–0.5	*Myco. phlei*	0.05–0.078
B. subtilis	0.02–0.1	*Myco. tuberculosis*	<0.5
Br. abortus	1.25–5.0	*Myco. tuberculosis* R	<0.5
Br. melitensis	0.625–2.5	*Neis. intracellularis*	1.25–2.5
Br. suis	0.312–2.5	*Past. pestis*	0.625
Clos. perfringens	>10.0	*Past. tularensis*	0.156
Cor. diphtheriae	0.156	*Phyt. pruni*	0.1
*_E. coli_	1.5–5.0	*Pr. vulgaris*	1.25–2.5

* Streptomycin-resistant

E. coli	1.25–2.5	*Ps. aeruginosa*	12.5–25.0
H. influenzae	1.25–2.5	*Sal. typhosa*	0.1–0.625
H. pertussis	2.5	*Sal. schottmuelleri*	0.4–0.7
K. pneumoniae	0.312–0.625	*Sar. lutea*	2.5
K. pneumoniae R	0.312	*Ser. marcescens*	1.25
Shig. paradysenteriae	0.25–0.5	*Strep. fecalis*	5.0
Staph. aureus	0.156–0.625	*V. comma*	2.5
Various fungi	>10.0		

Inhibition concentration (dilution units per mg of crude neomycin)[1]:

B. subtilis	150–750	Bodenheimer's org.	15
B. mycoides	20–150	*Ser. marcescens*	20
B. cereus	20–60	*Mycobacterium*	50–250
Staph. aureus	100–250	*Trich. mentagraphytes*	<0.3
Ps. aeruginosa	2.5	*Candida albicans*	<0.3
**E. coli*	20	***E. coli*	25
Pr. vulgaris	25		

* Streptomycin-resistant ** Streptomycin-sensitive.

Factors affecting activity. Increase of activity in alkaline medium, reduced by glucose, slight loss by cysteine.[2]

Mode of action. Bacteriostatic and bactericidal.[2]

Resistance. Resistant strains not readily developed.[2]

Spectrum in vivo. In mice, neomycin administered subcutaneously gave 100% protection against experimental infections with *Staph. aureus* at 3.1–12.5 units; *Sal. schottmuelleri* at 75 units; 50–90% protection against *Sal. pullorum* at 100 units; 90% protection against *E. typhosa* at 100 units. Oral administration of 800 units gave complete protection against *Staph. aureus*.[1, 4]

In chick embryos, 50 units afforded 100% protection against *Staph. aureus*, 100–200 units gave from 40–100% protection against *Sal. schottmuelleri*, 100–280 protected 50–90% against *Sal. pullorum*.[1, 4]

Synergism. Between neomycin and streptomycin in *Sal. pullorum* infections in chick embryos.[4]

PHARMACOLOGY

Toxicity. In mice, administered subcutaneously, 2,000–5,000 units were tolerated; mild transient irritation by neomycin at 1,500 units per ml instilled into the eye sac of rabbit; no evidence of delayed toxicity over 7 days.[4]

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NEOMYCIN A *See* NEOMYCIN

NISIN

First described by Mattick and Hirsch in 1944[1]; derived from lactic streptococci group N.

PRODUCTION

Fermentation. On medium containing g/l glucose 15, "Yeastrel" 7, sodium citrate 6, K_2HPO_4 10, KH_2PO_4 10, $MgSO_4$ 0.2, NaCl 3, pH 7.2; incubated at 30° C for 48 hours; final pH 4.4; yield 80 *St. agalactiae* units/ml.[2]

Isolation. Culture liquor acidified with HCl to pH 2 and centrifuged; the supernatant liquor adjusted to pH 4.2–4.5 with NaOH and extracted with chloroform containing 2% secondary octyl alcohol; the chloroform extract cooled to 4° C and active material precipitated with cold absolute alcohol; the precipitate dissolved in dilute HCl, the pH adjusted to 2.5–3.0 with NaOH, an equal volume of cold alcohol added to precipitate-inactive material; the supernatant concentrated in vacuo, the pH adjusted to 4.0 and the solution cooled; the precipitate removed, washed with distilled water, dissolved in dilute HCl and freeze-dried; yield about 20% of activity of culture liquor.[2]

Purification. Crude nisin dissolved in dilute HCl at pH 1.8 in molar phosphate buffer, the pH adjusted to 6–1 with KOH, the precipitate removed and dissolved in dilute HCl at pH 1.9; then fractionally precipitated with sodium chloride; the wet amorphous precipitate dissolved in absolute ethanol, crystallized by slow cooling.[3]

CHEMISTRY

Properties. Long needle-like crystals[3]; thermostable in acid solution.[2]

BACTERIOLOGY

Assay. Serial dilution with *St. agalactiae;* crystalline nisin 50,000–100,000 units/mg.[3]

Spectrum in vitro. Inhibition concentration (units/ml)[2]:

Pneumococcus I	0.25	*Clostridium* sp.	0.25–80
Pneumococcus II	100	*Corynebacterium* sp.	4–120
Staph. pyogenes	100	*Actinomyces* sp.	1–10
Neisseria sp.	2–50	*Myco. tuberculosis*	100–500
Bacillus sp.	2–4	*Erysip. monocytogenes*	2
Streptococcus A–M (except C, D, L)	0.25–4		

Active against *M. lysodeikticus*, lactobacillus; no activity against gram-negative forms.[2]

Factors affecting activity. Activity is unaffected by serum, blood or milk.[2]

Spectrum in vivo. Mice, experimentally infected with a mouse-pathogenic streptococcus, were protected by 50 mg (100,000 units) of nisin administered 12 hours after infection. Nisin also protected mice against experimental infection with *Cl. septicum* and *St. pneumoniae* II; in guinea pigs, tuberculosis infections were somewhat inhibited.[2]

PHARMACOLOGY

Toxicity. In mice, LD 50 = 3.5 g/kg; no chronic toxicity; no effect on leucocytes, no hemolysis of red cells at 1:250.[2]

Blood level, excretion. In rabbits, 200,000 units intramuscularly produced 4 units/ml in the blood; activity was detected in the urine of rabbits and guinea pigs.[2]

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NOCARDIN

First described by Emmart in 1917 [1]; derived from *Nocardia coeliaca*.

PRODUCTION

Fermentation. Surface growth on medium containing g/l Na_2HPO_4 3.0, KH_2PO_4 4.0, $MgSO_4$ 0.6, sodium citrate 2.5, asparagin 5.0, glycerin 20, sucrose or trehalose 20; incubated at 27.5° C for 61 days.[1]

Isolation. *From culture filtrate.* Activity adsorbed on activated carbon, eluted with ether-alcohol or acid alcohol; eluate evaporated to dryness. *From mycelium.* Extraction with ether and 95% alcohol.[1, 2]

BACTERIOLOGY

Assay. Serial dilution with *Myco. tuberculosis.*[1]

Spectrum in vitro. Crude nocardin active against several *Myco. tuberculosis* strains.[1]

Spectrum in vivo. In mice, experimentally infected with *Myco. tuberculosis*, 25 mg twice daily of crude nocardin administered subcutaneously, caused approximately 50% reduction in tuberculous lesions over untreated controls.[2]

In guinea pigs, evidence of protection against *Myco. tuberculosis;* inhibition of tuberculosis in chick embryo by crude nocardin.[1]

PHARMACOLOGY

Toxicity. In mice, 50 mg twice daily for one week of crude nocardin administered subcutaneously, was approximately 50% lethal.[2]

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NOTALIN *See* CITRININ

NOTATIN *See* PENATIN

OBTUSIN

First described by Robbins, Kavanagh, and Hervey in 1945; derived from a *Basidiomycetes* sp.

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OXYASIATICOSIDE *See* ASIATICOSIDE

OXYJAVANICIN *See* JAVANICIN

α-OXYPHENAZINE *See* PYO COMPOUNDS

PARASITICIN *See* PENICILLIN

PATULIN *See* CLAVACIN

PENATIN

First described by Kocholaty in 1942[1] and by Coulthard, Michaelis and Short as "PENICILLIN A" in 1941[2]; name changed to NOTATIN by Coulthard *et al.* in 1942[3]; also known as PENICILLIN B[4] (identity of penatin, notatin and penicillin B),[5] as E. COLI FACTOR,[6] as P.L. or CORYLOPHYLIN [7, 8, 9, 10]; derived from *Penicillium notatum;* also from *P. resticulosum* [11] and *P. corylophylum.*[9]

PRODUCTION

Fermentation. Surface growth on (A) Modified Czapek-Dox with manganese 10 mg/l (penatin formation inhibited if corn-steep liquor, brown sugar, yeast extract or malt extract added to culture medium, and another antibiotic formed); incubated at 28° C for 7 days; final pH 3.5–4.0.[12, 13, 14] (B) Czapek-Dox at pH 6.0–6.5; final pH 3.5; yield 70,000 *Staph. aureus* dilution units/ml.[3, 11] (C) Czapek-Dox with g/l sodium acetate·$3H_2O$ 1.0, trisodium citrate·$3H_2O$ 1.0, $CuSO_4 \cdot 5H_2O$ 0.0175, pH 7.8; incubated at 20–22° C for 16 days; yield 10,000–25,000 *Staph. aureus* dilution units/ml.[11] (D) Medium containing g/l $NaNO_3$ 3.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, glucose 40.0, pH 4.5; incubated at 24° C for 12 days, final pH 4.0; yield 1,250–5,000 *Staph. aureus* dilution units/ml.[4]

Isolation. (A) Precipitation with phosphotungstic acid.[4] (B) Culture filtrate activity adsorbed on kaolin at pH 3.5–4.0, the kaolin washed with water and the activity eluted with phosphate buffer at pH 6.3 or with pyridine; penatin precipitated from the eluate with dioxan, the precipitate dissolved in water and freeze-dried; yield 10–50%.[13, 14] (C) Culture filtrate concentrated *in vacuo,* chilled and acetone added; the precipitate removed, dissolved in water and reprecipitated from cold acetone.[3, 11, 15] (D) Culture filtrate activity precipitated with tannic acid, followed by extraction with acetone.[3, 15] (E) Chilled culture filtrate adjusted to pH 3.5 with phosphoric acid, saturated benzoic acid in alcohol added, 50 ml per liter, stirred and filtered; the moist precipitate washed with cold water saturated with benzoic acid, extracted with cold acetone, the residue washed with acetone and dried *in vacuo.*[4] (F) Culture filtrate activity precipitated with uranium acetate at pH 3.5–3.9, the precipitate extracted with pH 6.8 phosphate buffer; penatin salted out of the buffer extract with ammonium sulfate, centrifuged, dissolved in water, dialyzed until sulfate-free and freeze-dried.[16]

Purification. (A) Dialysis against distilled water.[11, 13] (B) Precipitation with tannic acid, followed by repeated extraction and precipitation from acetone; yield 30–40%.[11] (C) Precipitated with Reinecke's salt, decomposed with acetic acid and precipitated from cold acetone.[11]

BACTERIOLOGY

Assay. Serial dilution with *Myco. tuberculosis.*[1]

Spectrum in vitro. Crude nocardin active against several *Myco. tuberculosis* strains.[1]

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Isolation. (A) Precipitation with phosphotungstic acid.[4] (B) Culture filtrate activity adsorbed on kaolin at pH 3.5–4.0, the kaolin washed with water and the activity eluted with phosphate buffer at pH 6.3 or with pyridine; penatin precipitated from the eluate with dioxan, the precipitate dissolved in water and freeze-dried; yield 10–50%.[13, 14] (C) Culture filtrate concentrated *in vacuo,* chilled and acetone added; the precipitate removed, dissolved in water and reprecipitated from cold acetone.[3, 11, 15] (D) Culture filtrate activity precipitated with tannic acid, followed by extraction with acetone.[3, 15] (E) Chilled culture filtrate adjusted to pH 3.5 with phosphoric acid, saturated benzoic acid in alcohol added, 50 ml per liter, stirred and filtered; the moist precipitate washed with cold water saturated with benzoic acid, extracted with cold acetone, the residue washed with acetone and dried *in vacuo.*[4] (F) Culture filtrate activity precipitated with uranium acetate at pH 3.5–3.9, the precipitate extracted with pH 6.8 phosphate buffer; penatin salted out of the buffer extract with ammonium sulfate, centrifuged, dissolved in water, dialyzed until sulfate-free and freeze-dried.[16]

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(D) Fractional precipitation or extraction with acetone or with ammonium sulfate.[4, 11, 16] (E) Adsorption on aluminum hydroxide or tricalcium phosphate gel.[11] (F) Chromatography on aluminum oxide, elution with pH 5.0 phosphate buffer.[11]

CHEMISTRY

Constitution. Flavoprotein, glucose-oxidase enzyme[3, 5, 11, 17, 18]; by analysis C 50.0–52.5, H 6.7–7.2, N 11.8–14.2, P 0.17–0.58[3, 11]; N 9.7, 8.2,[4] N 14[16]; M.W. 152,000 (ultracentrifuge),[19] equivalent weight 83,000 (absorption bands of flavoproteins).[18]

Properties. Light-yellow or buff powder[3, 13, 14]; soluble in water, insoluble in organic solvents[3, 4, 13]; nondialyzable through cellophane[4, 13]; ultraviolet maxima at 280, 375, 465 mμ,[3] maximum at 277 mμ, minimum at 277 mμ,[4] maxima at 270–280, 375–380, 450–460 with $E^{1\%}_{1\text{cm}} = 1.54$ (assuming M.W. 73,000),[11] maxima 377, 455[18]; $[\alpha]_D^{22°} = -4.8°$ (c = 0.0125)[11]; salted out by ammonium sulfate or magnesium sulfate[4]; adsorbed by alumina gel or "Decalso," eluted with ammonium sulfate or acetate[4]; adsorbed on fuller's earth.[13]

Reactions. Positive protein reactions; unstable above and below pH 2–8; heat-labile[3, 4, 11, 13]; inactivated by glacial acetic acid, formic acid, concentrated phthalate buffer, alcohol with high salt concentration, low concentrations of acetone and by methanol, but not by ethanol[4, 13]; precipitated by tannic, tungstic, phosphotungstic, phosphomolybdic, trichloroacetic, flavianic and rufianic acids[4, 13]; not digested by pepsin, trypsin, "take-diastase," emulsin[4, 11]; inactivated by papain-HCN[4]; green fluorescence with acetic acid.[11]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* agar streak with *E. coli* or *Staph. aureus.*[13] Purified penatin: inhibition dilution 1:6 billion,[16] 12,500–200,000 units/mg,[4] 300–400 × 10^6 units/mg.[11]

Spectrum in vitro. Inhibition dilution (× 10^6)[11, 13, 14]:

Staph. aureus	42–125	*N. catarrhalis*	250
Staph. albus	125	*C. diphtheriae*	42
St. hemolyticus	10–1,000	*G. tetragena*	250
B. subtilis	25	*Eb. typhi*	42–1,000
B. anthracis	10–25	*Br. melitensis*	25
B. mycoides	25	*Shig. paradysenteriae*	100
B. brevis	42	*Shig. sonnei*	1
Sar. lutea	250	*Aero. aerogenes*	25
D. pneumoniae	12.5–100	*Br. abortus*	10–25
E. coli	1–12.5	*Ps. aeruginosa*	42

S. paratyphi	25–100	*Pr. vulgaris*	100–1,000
S. schottmuelleri	10	*K. pneumoniae*	0.1–1
S. typhimurium	10	*V. comma*	1,000
S. enteriditis	10	*Cl. histolyticum*	42
S. cholera-suis	0.01–0.1	*Streptomyces lavendulae*	42
		Trich. interdigitale	12.5

Inhibition concentration (p.p.m.) [4]:

E. coli	0.8–3.2	*S. schottmuelleri*	3.2
Aero. aerogenes	1.6–3.2	*S. enteriditis*	6.4
Pr. vulgaris	12.8	*S. paratyphi*	12.8
Staph. aureus	51.2	*Eb. typhi*	12.8
B. subtilis	12.8	*V. comma*	12.8
Br. abortus	6.4	*Br. suis*	12.8
Br. melitensis	12.8	*D. pneumoniae*	0.8

At a dilution of 25–50 × 10^6, penatin inhibited 3 molds, 4 actinomycetes and 16 soil bacteria [13]; inhibited anti-coliphage and amoebae.[20, 21]

Factors affecting activity. Maximum activity in the presence of glucose 0.5% or over and of oxygen [11]; not antagonized by serum [13, 14]; inactivated by blood and serum, not by heated blood [22]; antagonized by catalase.[11]

Mode of action. Bacteriostatic [13]; activity derived from hydrogen peroxide produced by enzyme action of penatin [3, 5, 11, 16, 17, 21]:

$$\text{glucose} + H_2O + O_2 \xrightarrow{\text{penatin}} \text{gluconic acid} + H_2O_2$$

Spectrum in vivo. In guinea pigs with experimental *Br. abortus* infections, treated subcutaneously with penatin in repeated small doses, showed gain in weight as compared with untreated controls.[21] In mice, penatin failed to protect against *St. hemolyticus*, *Staph. aureus* and *Salmonella* infections.[22] No effect on the Rous sarcoma virus.[23]

PHARMACOLOGY

Toxicity. In mice, LD 50 mg/kg: intraperitoneal 3, subcutaneous 4.5, intravenous [13]; in rabbits, subcutaneous, 7.5; oral administration had no effect at 300 mg/kg.[22] In guinea pigs, 500 mg intramuscularly was lethal, 10–20 mg/day for 5 days was tolerated; in rabbits, intravenously, 16.5 mg was tolerated [13]; no marked chronic toxicity in rats [22]; methemoglobin formed in rats and anaesthetized rabbits, darkening of blood in unanaesthetized rabbits.[22] Penatin administered subcutaneously caused local edema, swelling, adhesions and subcutaneous hemorrhage.[22] Toxicity increased with antibacterial potency.[22] At 1:200, did not inhibit phagocytosis.[22]

Physiology. Penatin produced contraction of isolated smooth muscle at 1:40,000–200,000, vasoconstriction of the rabbit ear, and an antidiuretic effect.[22]

MISCELLANEOUS

Penatin has been used to estimate glucose in enzyme systems and biological kinetics by manometry of oxygen.[24]

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PENICIDIN

First described by Atkinson in 1942; derived from a *Penicillium* sp.

PRODUCTION

Fermentation. Surface culture on modified Czapek-Dox medium at 18-20° C, optimum yield at about 5 days, culture liquor may be drained from mycelium and replaced with fresh medium to give additional yield 3-4 days after replacement.

Isolation. Culture filtrate concentrated in vacuo, adjusted to pH 7.0, extracted with ether, ether extract shaken with water adjusted to pH 4.0 with sulfuric acid; aqueous extracts neutralized, extracted with ether; ether extract evaporated in vacuo and petroleum ether added; crude penicidin separates out as a pale yellow oily layer.

CHEMISTRY

Properties. Thermostable at pH 7.0, stable at pH 3.0-7.8; unstable in highly alkaline solutions; soluble in chloroform, benzene, ether, ethanol, dilute mineral acids; insoluble in petroleum ether; dialyzes readily through cellophane membranes; adsorbed by activated carbon.

Reactions. Negative biuret, Molisch, Millon; in NaOH solution, produces yellow color and yellow fluorescence under ultraviolet; faint purplish-brown with slight excess ferric chloride, disappearing with additional ferric chloride.

BACTERIOLOGY

Inhibited *Eb. typhi* at 1:100,000; active against both gram-negative and -positive bacteria.

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PENICILLIC ACID

First described by Alsberg and Black in 1913 [1]; derived from *Penicillium puberulum*,[1] *P. cyclopium*,[2, 3] *P. thomii*, *P. suavolens*,[4] *Aspergillus ochraceus*,[4] *A. quercinus*, *A. sulphureus*,[5] *A. melleus*,[10] and by synthesis.[6]

PRODUCTION

Fermentation. With *P. puberulum* and *P. cyclopium* surface growth on medium containing g/l glucose 75, tartaric acid 4, diammonium tartrate 4, NH_4NO_3 4, $(NH_4)_2HPO_4$ 0.6, K_2CO_3 0.6, $MgCO_3$ 0.4, $(NH_4)_2SO_4$ 0.25, $ZnSO_4 \cdot 7H_2O$ 0.07, $FeSO_4 \cdot 7H_2O$ 0.07; incubated at 24° C for 19–23 days; yield 2.26 g/l.[2, 3]

With *A. ochraceus* on Czapek-Dox medium with corn-steep liquor 3%; incubated for 3 days.[4]

Isolation. (A) Culture filtrate acidified with HCl and extracted with chloroform, the chloroform extract washed with water, dried over anhydrous magnesium sulfate and evaporated *in vacuo* to dryness.[3] (B) Culture filtrate evaporated *in vacuo*, acidified and extracted with ether.[2] (C) Culture filtrate activity adsorbed on activated carbon at pH 7.0, eluted with methanol and evaporated *in vacuo* to a crystalline mass.[4] (D) Culture filtrate acidified to pH 2, extracted with ether; the ether extract evaporated to dryness.[4]

Purification. (A) Treatment with activated carbon and recrystallization from ether.[2] (B) Recrystallization from 50% methanol.[4] (C) Sublimation in high vacuum at 75° C.[3]

Synthesis. Lactone plus N-bromosuccinimide yields bromolactone; the latter warmed with triethylamine gives quaternary lactone; the latter boiled in water with excess magnesium oxide forms penicillic acid.[6]

CHEMISTRY

Constitution. $C_8H_{10}O_4$ (crystallized from petroleum ether — anhydrous form), $C_8H_{12}O_5$ (crystallized from water — hydrated form) [1, 2]; γ-keto-β-methoxy-Δ^{α}-hexanoic acid or the tautomeric corresponding $\Delta^{\alpha\beta}$-γ-hydroxy-lactone.[2]

Properties. Large colorless rhombic or hexagonal plates [2]; m.p. 64–65° C,[2] 61° C,[4] 86–87° C [6]; soluble in water (2%), alcohol, ether, benzene, chloroform; insoluble in petroleum ether.[2]

Reactions. Orange-brown color when heated with ferric chloride.[2]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus* and *E. coli*.[3]

Spectrum in vitro. Inhibition dilution (× 10,000)[7]:

B. anthracis	10	*Eb. typhi*	3.3
E. coli	50	*S. typhimurium*	3.3
Sh. dysenteriae	5–10	*Staph. albus*	10
S. enteriditis	3.3	*Staph. aureus*	<2–5
Pr. vulgaris	5	*Staph. citreus*	<2
S. paratyphi	3.3	*St. viridans*	<1.2
V. comma	10		

Factors affecting activity. Not affected by pH; antagonized by peptones, nutrient broth, amines and amino acids.[8]

PHARMACOLOGY

Toxicity. In mice, LD 50 mg/20 g: subcutaneous 2.2, intravenous 5, oral 12.[9]

Physiology. Digitalis-like action on frog heart, rabbit auricle, cat heart; weak action on heart-lung preparation of dog. Dilator effect on systemic, coronary and pulmonary blood vessels. In intact cat, initial rise of blood pressure followed by prolonged fall. In rats, antidiuretic effect possibly due to diminished permeability of glomerular capillaries.[9]

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PENICILLIN

(The following is a summary in extremely abbreviated form of the basic information relating to penicillin; see Introduction, page 4.)

First described by Fleming [*Brit. J. Exper. Path.* **10**, 226 (1929)], derived from *Penicillium notatum*, from other *Penicillia* and from *Aspergilli.*

Several types of penicillin have been identified:

F — I	2-pentenyl penicillin
dihydro F — dihydro I	*n*-amyl penicillin
G — II	benzyl penicillin
X — III	*p*-hydroxybenzyl penicillin
K — IV	*n*-heptyl penicillin

GIGANTIC ACID has been identified as *n*-amyl penicillin, PARASITICIN as benzyl penicillin, FLAVACIN (FLAVICIDIN or FLAVATIN) as a mixture of benzyl, 2-pentenyl and *n*-amyl penicillins.

PRODUCTION

Fermentation:

Methods. Surface growth in flasks, bottles or trays. Submerged growth in shaker flasks, stirred aerated tanks and rotary fermenters, on bran, on cellophane and by percolation through wood shavings.

Media. Nitrogen sources: corn-steep liquor, casein, cottonseed meal, peanut meal, distiller's wastes or as nitrate or ammonium salts. Carbon sources: lactose, starch, dextrin, glucose, maltose, molasses. Inorganic ions: zinc, copper, manganese, potassium, sodium, calcium, magnesium, phosphate, sulfate and nitrate. Precursors for benzyl penicillin: phenylacetic acid and derivatives.

Incubation temperature. 25–28° C.

Incubation time. Surface growth for 7 days; submerged growth 2–3 days.

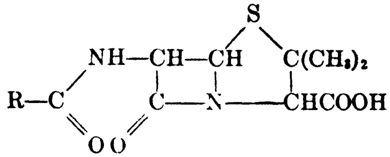
Yields. Surface growth, over 200 units/ml; submerged growth up to 1,000 units/ml or over.

Isolation. (A) Carbon process: adsorption on activated carbon, elution with water and an organic solvent such as acetone, amyl acetate or chloroform. (B) Solvent process: extraction of acidified culture liquor with organic solvent such as amyl acetate, chloroform, ether or isopropyl acetate; extraction from organic solvent by buffer at pH 7 or with dilute alkali. (C) Ion-exchange resin process.

Purification. Chromatography; crystallization of alkali salt from organic solvent.

CHEMISTRY

Constitution:



R	Penicillin type
2 pentenyl	F
n-amyl	dihydro F
benzyl	G
p-hydroxybenzyl	X
n-heptyl	K

Properties. Strong monobasic acid; free acid soluble in organic solvents and slightly soluble in water. Salts with alkali metals and alkaline earths very soluble in water.

Reactions. Unstable in aqueous solution; decomposed rapidly by acids and alkalies, and by penicillinase.

BACTERIOLOGY

Assay. Cylinder-plate or turbidimetric with *Staph. aureus*, most commonly employed; colorimetric chemical assay. Units/mg of sodium salt: F 1,625, dihydro F 1,610, X 970, G 1,667, K 2,300.

Spectrum in vitro. Very active against most gram-positive bacteria, against pathogenic *Neisseriae*, *Treponemata* and some species of *Hemophilus* over a range of 0.001–1.0 γ/ml. Less active against gram-negative forms including the colon-typhoid group, *Brucella*, actinomyces and most spirochetes. Inactive against *Myco. tuberculosis*, fungi, yeasts, viruses, rickettsiae and protozoa, and against resistant strains of susceptible organisms.

Factors affecting activity. Activity unaffected by serum, pus or tissue fluid; antagonized by penicillinase-producing bacteria and by amino acids.

Synergism. Increased activity with paraaminobenzoic acid, sulfonamides and many other antibacterial compounds.

Resistance. Frequent occurrence of natural and acquired resistance among strains of susceptible organisms.

Mode of action. Bacteriostatic, slowly bactericidal; more active against young, rapidly multiplying bacterial cells.

Spectrum in vivo. Effective by parenteral administration in systemic infection with susceptible organisms in animals and plants, and clinically; less effective by oral or rectal administration. Effective by aerosol administration; and by topical application in ointment form for superficial infections.

Toxicity. The toxicity of penicillin is related to the cation forming the salt, and to the degree of purity of the penicillin. The pure salt with sodium, least toxic of all the cations tested, administered intravenously to laboratory animals, has an LD 50 of 1–2 g/kg, or approximately 100 times the average therapeutic dose.

Clinically, allergic and shock reactions have been reported as resulting from penicillin administration.

Blood levels. Therapeutic blood levels were maintained in the blood for 1–2 hours after administration of 20,000–100,000 units parenterally; blood levels maintained for longer periods, without repeated administration, by prolonging diffusion (*e.g.*, peanut oil-beeswax, procaine penicillin) or by prolonging excretion (*e.g.*, para-aminohippuric acid, diodrast, caronamide).

Spinal fluid levels. Little or no penicillin was detected in the spinal fluid following parenteral administration.

Fetal blood levels. Significant blood levels found in the placenta following administration into maternal blood stream.

Excretion. Penicillin was rapidly excreted, about one-third of the administered dose being recovered in the urine.

Absorption. Administered orally, about two-thirds of the penicillin was destroyed in the intestinal tract or eliminated with the feces; the remainder was rapidly absorbed. Various penicillin preparations, including antiacids and penicillin esters, have been proposed for oral administration.

Diffusion. Penicillin diffused readily into organs, tissues and body fluids with the exception of the brain.

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PENICILLIN A *See* PENATIN

PENICILLIN B *See* PENATIN

PHENICIN

First isolated by Friedheim and described as a respiratory pigment, in 1933 [1]; antibiotic activity first described by Burton in 1949 [2]; also known as PHOENICIN [2]; derived from *Penicillium phoeniceum* [1,3] and *P. rubrum.*[2]

PRODUCTION

Fermentation. Surface growth on medium containing g/l peptone 10, malt extract 26, glucose 40; incubated for 8 days.[2]

Isolation. Acidified culture filtrate extracted with chloroform; chloroform extract shaken with sodium bicarbonate solution to yield a deep violet extract.[2]

Purification. Chloroform solution chromatographed over alumina.[2]

CHEMISTRY

Constitution. Proposed $C_{14}H_{10}O_6$ [2,4,5]; 2,2′-dihydroxy-4,4′-dimethylbiphenyl-3,6,3′,6′-diquinone, confirmed by synthesis [4,5]; calculated C 60.6, H 4.02, (C)CH_3 10.9, found C 58.61, H 4.17, (C)CH_3 9.7, active H 1.03.[2]

Properties. Yellow-brown crystals,[2] long rectangular tablets [4,5]; m.p. 229–230° C,[2] 230–231° C [4,5]; soluble in chloroform, acetic acid, warm ethanol; slightly soluble in water [2]; yellow, red or violet color varying with pH.[4]

Reactions. A dibasic acid [4,5]; released iodine from potassium iodide, decolorized by sodium hydrosulfite.[2]

BACTERIOLOGY

Active against *Staph. aureus*, *Coryne. xerosis* and *Myco. phlei.*[2]

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PHTHIOCOL

Antibiotic activity first described by Lichstein and Van de Sand in 1946 [1]; derived from *Mycobacterium tuberculosis* and named by Anderson and Newman in 1933.[2] Phthiocol is an isomer of PLUMBAGIN.

PRODUCTION

M. tuberculosis culture extracted with acetone; the acetone-soluble fraction saponified and extracted with ether.[2]

CHEMISTRY

Constitution. 2-methyl-3-hydroxy-1,4-naphthoquinone.[2]

Properties. Yellow prisms; m.p. 173–174° C, volatile with steam; soluble in common organic solvents; insoluble in water and petroleum ether.[2]

Reactions. Deep-red salts with alkalies and strong bases, soluble in water, alcohol and acetone.[2]

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (per cent) [1]:

St. pyogenes	0.02	*C. diphtheriae*	0.05
D. pneumoniae	0.02	*E. coli*	0.05
B. anthracis	0.02	*Sh. paradysenteriae*	0.05
Staph. aureus	0.05	*Eb. typhi*	0.1
St. salivarius	0.05	*N. catarrhalis*	0.1
Ps. aeruginosa	0.1		

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P. L. See PENATIN

PLEURIN

First described by Robbins, Kavanagh and Hervey in 1945; derived from a *Basidiomycetes* sp.

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PLEUROTIN

First described by Robbins, Kavanagh, and Hervey in 1947[1]; derived from *Pleurotus griseus.*

PRODUCTION

Fermentation. Surface growth on beechwood shavings in medium containing g/l $NaNO_3$ 3.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7\,H_2O$ 0.5, $Fe_2(SO_4)_3$ 0.01, dextrose 40, corn-steep liquor solids 5, incubated at 25° C for about one month; yield approximately 1,000 *Staph. aureus* units/ml; additional yields obtained by repeated reflooding of the mycelium with fresh medium.[1]

Isolation. (A) Culture filtrate extracted with chloroform, the resulting emulsion centrifuged; the chloroform layer washed with 1% sodium bicarbonate, evaporated in vacuo to a gummy residue; the residue dissolved in hot ethanol, ether added and the solution allowed to evaporate at room temperature to yield orange-colored crystalline pleurotin. (B) Activity adsorbed from culture liquor with activated carbon, eluted from air-dried carbon cake with chloroform.[1]

Purification. Recrystallization from chloroform-ether; yield 100–800 mg/l culture liquor.[1]

CHEMISTRY

Constitution. Proposed: $C_{20}H_{22}O_6$, C 70.83, H 6.43 and M.W. 342.4; M.W. (Rast) 343.[1]

Properties. Orange crystals from ethanol-ether; large amber crystals by slow evaporation, fine yellow needles by rapid evaporation of chloroform-ether; m.p. 200–215° C (dec.). Soluble mg/ml at 25° C: water 0.125, 95% ethanol 6.8, 5% ethanol 0.37, ether 3.5, chloroform >200; soluble in acetone; relatively insoluble in dilute acids, dilute sodium carbonate, petroleum ether; $[\alpha]_D^{23} = -20°$ in chloroform ($c = 0.59$); ultraviolet maximum at 2,500 Å, molecular extinction coefficient 13.68 in 4% ethanol; neutral reaction in solution; thermolabile, particularly above pH 8.5; inactivated by light; no loss by "Seitz" filtration.[1]

Reactions. Reacts with alkali to give acidic product without activity; no color with ferric chloride; liberates iodine from acidified KI; blue color with KCN, reaction product exhibits bright blue fluorescence under 3,660 Å lines of mercury arc.[1,2]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus* as test organism; no zones of inhibition on agar disc saturated with pleurotin.[1]

Spectrum in vitro. Inhibition concentration (γ/ml)[1,2,3]:

Staph. aureus	0.8	*Photo. fischeri*	6.0
B. mycoides	1.6–3	*Myco. phlei*	>32
B. subtilis	0.2	*Myco. smegmatis*	32
E. coli	500–>500	*K. pneumoniae*	500–>500
Myco. tuberculosis H234	>100		

PHARMACOLOGY

In mice, 24 mg/kg intravenously was non-toxic; larger doses not possible due to low solubility in water.[1]

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PLUMBAGIN

Used medicinally in India under the name "Chita"; derived from the bark of roots of *Plumbago europaea*, *P. zeylonica*, *P. rosea*, and by synthesis.[1,2] Plumbagin is an isomer of PHTHIOCOL.

PRODUCTION

Extraction from *Plumbago* sp.

Synthesis from *m*-toluyl chloride and acetylsuccinic ester,[1] and by oxidation of 2-methyl-1,4-naphthoquinone with Caro's acid.[2]

CHEMISTRY

Constitution. 2-methyl-5-hydroxy-1,4-naphthoquinone.[1]

Properties. Orange-yellow pigment; m.p. 78–79° C[1,2]; very soluble in organic solvents, slightly soluble in water.[3]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 10,000)[3]:

Staphylococcus	10–50	*Eb. typhi*	1–5
St. hemolyticus	10–50	*E. coli*	1–5
Pneumococcus	10–50	*Myco. tuberculosis*	5–10
B. subtilis	<0.3		

At 1:50,000, inhibited *Phialophora verrucosa*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Ctenomyces radians*, *Trichophyton ferrugineum*.[4]

Mode of action. Bacteriostatic and lytic.[3]

Spectrum in vivo. Clinically, good results with intravenous administration, for therapy of *Staphylococcus* infections causing furuncles and acne.[5]

PHARMACOLOGY

Toxicity. Lethal action on respiratory system of rats, guinea pigs and frogs [2]; low toxicity in vivo.[4]

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POLYMYXIN

Polymyxin is a generic name for the various antibiotics derived from strains of *Bacillus polymyxa*, syn. *B. aerosporus;* syn. AEROSPORIN, BACILLOSPORIN; discovered independently and published in 1947 practically simultaneously by Benedict and Langlykke,[1] Stansly, Shepherd and White [2] and Ainsworth, Brown and Brownlee.[3] At present, five polymyxin types have been isolated [4, 5]:

Polymyxin	Described by	Synonym
A	Ainsworth, Brown and Brownlee [3]	aerosporin, bacillosporin A
B	Jones [5]	bacillosporin B
C	Jones [5]	bacillosporin C
D	Stansly, Shepherd and White [2]	polymyxin
E	Jones [6]	

The polymyxin of Benedict and Langlykke [1] and of Reese and Eisenberg [7] has not as yet been fitted into this classification.

PRODUCTION

Fermentation. Submerged culture: (A) in 200-gal. tanks, in medium containing corn-steep liquor 3%, dextrose 3%, pH 6.5 after sterilization; aeration ratio 0.7 vol. air per vol. medium per minute, rpm 135, at 26–28° C, fermentation time 64–66 hours, final pH 6.7; yield: 400 *E. coli* dilution streak-plate units [8]; (B) in shaker flasks and in aerated 22-liter flasks, medium containing solvent-extracted soybean meal ("Nutrisoy XXX")

0.5%, mineral salts, dextrose and calcium carbonate sterilized separately 1% each; at 25° C, yields: in shaker flasks up to 100 units/ml; in aerated 22-liter flasks 400 units/ml, using *Brucella bronchiseptica* as test organism and the unit *of* Stansly and Schlosser[9] *i.e.*, one *E. coli* unit = 8 γ/ml of a partially purified polymyxin D hydrochloride arbitrary standard.[8]

Other media described as producing polymyxin in submerged culture: synthetic with biotin[10]; with yeast extract, tryptone or peptone[1]; with yeast extract, mineral salts and dextrose[2]; with soybean oil meal[11]; with extracted peanut meal, ammonium citrate, ammonium phosphate or ammonium sulfate[12]; with dextrose or sucrose superior to lactose[12]; with hydrol.[12]

Surface culture with nutrient broth containing sucrose or dextrose and manganese[3]; with yeast extract, mineral salts and dextrose.[2]

Isolation. (A) Adsorption and elution with activated carbon by method used for streptomycin[3, 12]; mucoid material in culture liquor makes filtration impossible, charcoal method abandoned.[8]

(B) Isopropanol and ammonium sulfate added to culture liquor, alcohol layer separated, evaporated to dryness or used alcohol solution for purification; yield 50% of activity in culture liquor.[8]

(C) Culture liquor adjusted to pH 2 with sulfuric acid, heated at 90° C for 30 minutes and filtered; active portion adsorbed on cotton sodium succinate, eluted with dilute sulfuric acid; "Polar Yellow" added, precipitating polymyxin as "Polar Yellow" salt; precipitate washed with water, dried in vacuo; dry precipitate suspended in 85:15 acetone-methanol and triethylamine sulfate added, converting polymyxin into the methanol-insoluble sulfate; the polymyxin sulfate washed with acetone-methanol and with acetone, taken up in water and freeze-dried; yield about 75%.[31]

Purification. (A) Alcohol solution of polymyxin adsorbed on aluminum oxide column previously washed successively with 10% H_2SO_4, with distilled water, and with methanol; after adsorption, column washed with 80% methanol followed by 50% methanol; eluted by distilled water, and combined eluates adjusted to pH 10 with *N* NaOH; filtered, adjusted to pH 7 with *N* HCl, precipitated with picric acid, and the precipitate dissolved in acetone; acetone solution saturated with HCl gas, precipitating the hydrochloride; the polymyxin hydrochloride dissolved in distilled water, ammonia gas introduced and polymyxin crystallized by chilling, yielding polymyxin assaying approximately 10 Shigella units/mg. In a typical run where the picrate precipitation and decomposition were omitted, an overall yield of 21% was obtained and a product assaying 5.78 Shigella units/mg (one Shigella gallinarum unit is roughly equivalent to 200 streak-plate *E. coli* units).[8]

(B) Fractional precipitation as helianthate by adding helianthic acid in dilute aqueous pyridine; the precipitate suspended in methanol with

HCl or H_2SO_4, the precipitated helianthic acid filtered off, the polymyxin in the filtrate precipitated with anhydrous acetone; further purification by precipitation as the amorphous reineckate, and regeneration either with silver sulfate and barium chloride or by suspending in dilute HCl, extracting the free reinecke acid with cyclohexane.[13, 14]

(C) "Polar Yellow"-purified polymyxin sulfate dissolved in water with addition of triethylamine at 0° C to precipitate free base; precipitate washed with ice water and with ether, suspended in water, dissolved by addition of sulfuric acid to pH 2.5 and warming, neutralized with barium hydroxide; filtrate freeze-dried; yield 85%.[31] Polymyxin-free base converted to crystalline polymyxin naphthalene-β-sulfonate[15] and recrystallized from aqueous ethanol; converted to hydrochloride with anhydrous methanol and concentrated HCl, precipitated from acetone.[31]

CHEMISTRY

Constitution. A basic polypeptide containing *l*-α,γ-diaminobutyric acid, a fatty acid $C_9H_{18}O_2$ (identified as *d*-6-methyloctan-1-oic acid[15]) and the following amino acids[4]:

Polymyxin	*d*-leucine	Phenylalanine	*l*-threonine	*d*-serine
A	+	−	+	−
B	+	+	+	−
C	−	+	+	−
D	+	−	+	+

A cyclic structure is suggested with an empirical formula for the hydrochloride: $C_{60}H_{97}N_{15}O_{15}Cl_4$ [4, 5, 13, 16]:

	Calculated	Found
C	43.5	43.5, 45.5
H	7.8	7.0, 7.7
N	15.2	15.1, 15.8
O	23.2	24.0 (by difference)
Cl	10.3	10.5, 14.0
M. W.	1,144	1,150
Equiv. W.	286	275–290

Properties. *Polymyxin hydrochloride:* Birefringent crystals with no definite structure, m.p. 220–250° C (dec.)[8], 228–230° C,[16] 230–235° C (dec.)[13]; $[\alpha]^{20}_D = -42°$ in water[13], $[\alpha]^{25}_D = -75.7°$ ($c = 1\%$ in aqueous 7.5% ethanol),[31] $[\alpha]^{23}_D = -40°$ ($c = 1.05$ water),[16] characteristic spectra in infrared,[17] characteristic ultraviolet spectrum for polymyxins B and C with inflections characteristic of phenylalanine; no ultraviolet spectrum for polymyxin A and D.[13]

Soluble in water (more than 40%), methanol; less soluble in higher alcohols, partition coefficient between water and higher alcohols inversely proportional to the salt concentration of the aqueous phase and to the pH; insoluble in ether, esters, ketones, hydrocarbons, chlorinated solvents[16]; adsorbed on filter paper.[3, 18]

Reactions. *Polymyxin-free base:* Slightly soluble in water, almost insoluble in alcohol.[16] Positive biuret, ninhydrin; negative Molisch, Sakaguchi, Pauly, Hopkins-Cole; no sulfur, alkoxy, ash; alkali-labile, acid-stable; precipitates with picric, flavianic, helianthic, Reinecke acids; forms amorphous and sometimes glassy materials with acetic, phenylacetic, oxalic, malic, benzoic, phthalic, cinnamic, succinic and naphthalene β-sulfonic acids; birefringent base may be precipitated from concentrated aqueous solutions of the hydrochloride by saturation with ammonia[13, 16]; not digested with trypsin, pepsin or papain.[23]

BACTERIOLOGY

Assay. Agar streak-plate method with *E. coli*, with *Pasteurella multocida*, with *Shigella gallinarum;* cylinder-plate method with *Shigella gallinarum*[8]; agar dilution spot method[19]; serial dilution method with *Brucella bronchiseptica*[12]; serial dilution with *E. coli* and penicillin (to inhibit *B. polymyxin* in culture medium assays)[7, 20]; serial dilution with *S. typhi* for polymyxin in serum[3]; serial dilution with large inocula of *E. coli* for polymyxin in urine[3]; turbidimetric method with *E. coli*[12, 20]; paper disc-agar diffusion method with *E. coli*, with *Brucella bronchiseptica*[7, 21]; for polymyxin in blood and urine.[22]

Assay results affected by concentration of test organism[7, 19, 23, 24]; by assay medium, by penicillin in medium[7]; by serum[3]; plate assay zones increase at lower pH[18]; polymyxin adsorbed on filter discs,[18] and on Seitz filter pads.[12]

Units. Polymyxin A hydrochloride ("near" purity) 10,000 units/mg[20, 24]; polymyxin B potency similar to A[23]; polymyxin D hydrochloride pure 2,000 units/mg.[2]

The units of Polymyxin A and B are not related to those of Polymyxin D. Actually, on a weight basis, all three polymyxins are equally active. For practical convenience, the Polymyxin A and B units may be referred to as "English" or "Wellcome" units (as used in the Wellcome Physiological Research Laboratories, Beckenham, Kent, England), and the Polymyxin D unit as "American" or "Stamford" units (as used in the Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut), one "Stamford" unit being equivalent to 5 "Wellcome" units.

Spectrum in vitro. The antibacterial activity of Polymyxins A, B and D are very similar. The following tabulation summarizes the composite range of activity of the three polymyxins.

Inhibition Concentration (γ/ml)[8, 19, 23, 24, 25]:

E. coli	<0.04–0.3	*N. catarrhalis*	0.08
Eb. typhi	0.08–0.3	*K. pneumoniae*	0.2–5
S. paratyphi A	<0.04–0.5	*C. diphtheriae*	10
S. paratyphi B	0.08	*C. pyogenes*	20
S. typhimurium	0.64	*C. welchii*	20
S. enteriditis	0.64	Pleuropneumonia-like human	>80
S. cholera-suis	0.16	Pleuropneumonia-like bovine	>80
S. pullorum	0.75–3	*Staph. aureus*	>80
S. schottmuelleri	0.125–1.5	*St. pyogenes*	>80
Salmonella sp.	0.16–0.64	*St. agalactiae*	>80
Sh. dysenteriae	0.08–0.75	*St. fecalis*	>80
Sh. paradysenteriae	0.016–0.75	Streptococcus F	>20
Sh. sonnei	0.16–0.75	Streptococcus G	10
Sh. gallinarum	0.7	*St. viridans*	>80
Sh. schmitz	0.4–1.5	*Strep. pneumoniae* I	>20
Past. pestis	0.4	*Strep. pneumoniae* III	>80
Past. boviseptica	0.4	*N. gonorrheae*	>80
Past. muriseptica	0.25–0.45	*Myco. tuberculosis* avian	>80
Past. multocida	0.7	*Myco. tuberculosis* human	>80
Aero. aerogenes	0.2–0.45	*Myco. tuberculosis* bovine	>80
H. pertussis	0.04–0.25	*B. subtilis*	40
H. influenzae	0.02–0.3	*Er. rhusiopathiae*	>80
H. bronchisepticus	0.08–0.15	*Pr. vulgaris*	>80
V. comma	0.25–0.30	*Pr. morgani*	>80
Br. abortus	1.0–1.6	Pneumococcus	>64
Ps. aeruginosa	0.64–3	Meningococcus	>30

A. fumigatus	>20
Syn. racemosum	>20
Monilia albicans	>20
Trich. mentagrophytes	>20

Spectrum in vivo. *Experimental infections in animals. Chicks* infected with *Pasteurella multocida:* 72% protection with 2 mg (1,600 *E. coli* units) of crude Polymyxin D intraperitoneally, 0% control survival; with *Shigella gallinarum:* 2–14% protection with 2 ml of crude Polymyxin D broth intraperitoneally, 0% control survival.[8]

Mouse protection tests [8, 23, 26]:

Infection	Dose	Polymyxin	Administered	% Protection
Eb. typhi	0.5 mg a	D	intraperit.	100
	0.1 mg × 8 b	A	subcut.	100
	0.1 mg × 8 b	B	subcut.	72
	0.2 mg × 8 b	D	subcut.	92

K. pneumoniae	0.05 mg × 8 b	A	subcut.	50
	0.05 mg × 8 b	B	subcut.	33
	0.1 mg × 8 b	D	subcut.	33
	5 mg/kg c	D	intraperit.	100
H. pertussis	0.05 mg × 6 b	A		91
	0.1 mg × 6 b	B		100
	0.25 mg × 6 b	D		91
	5 mg/kg c	D	intraperit.	100
Pneumococcus	100 mg/kg c	D	intraperit.	0

a = 800 *E. coli* units/mg, b = A, B = 10,000 units/mg ("pure"), D = 2,000 units/mg ("pure"), c = units/mg not specified

Administration subcutaneously of Polymyxin: A 79% pure, D 77% pure [19, 20, 24]

Infection	Median effective dose (mg/kg)	Polymyxin	Relative % effectiveness
Past. multocida	0.53–0.59	A	100
	0.60–0.78	D	90
K. pneumoniae	0.26–0.35	A	100
	0.50–0.66	D	50
H. pertussis	6	A	100
	21	D	30

Infection	Dose	Polymyxin	Average survival rate, days	Control
H. pertussis	0.05 mg × 6 a	A	13.8	4.7
	0.5 mg × 6 b	D	14.2	4.7
	25–100 units c	A	9.3–21.0	4.7
Eb. typhi	0.1 mg × 6 a	A	6.6	0
	0.3 mg × 6 b	D	6.9	0
	50–100 units c	A	4.7–9.0	0
K. pneumoniae	0.1 mg × 3 a	A	3.8	0
	0.3 mg × 3 b	D	3.3	0
E. coli	25–100 units c	A	8.3–10	0.2
H. influenzae	50–100 units c	A	6.2–10.5	4.0
H. bronchisepticus	100 units c	A	6.0	0.8

a = "pure" Polymyxin A 10,000 units/mg, b = Polymyxin D 1,350 units/mg, c = "Wellcome" units

Some protection against *Ps. aeruginosa,* ineffective against *S. typhimurium, Strep. pyogenes, Strep. aureus, Er. rhusiopathiae, Strep. viridans.*[20] *Guinea pigs* infected with *Brucella suis* and with *Past. tularensis* showed no protection by treatment with polymyxin (type unclassified).[27]

Clinical tests. Polymyxin D, 70% "pure," at 3–7 mg/kg/day intramuscularly showed good results with infections caused by *Ps. aeruginosa,* *H. pertussis, A. aerogenes, K. pneumoniae, Br. suis* (acute); variable results with *Eb. typhi*.[28] Favorable results have been obtained with Polymyxin A in cases of *H. pertussis, E. coli* and *Ps. aeruginosa* infections, in typhoid carriers and in gastroenteritis [29, 30]; therapeutic dose 0.1 mg/kg/4 hours.[20]

Mode of action. Antibacterial action of polymyxin is bactericidal [19, 20, 25]; antibacterial action was extremely rapid, complete sterilization from less than 20 minutes to 1 hour under favorable conditions [19, 25]; *E. coli* showed partial lysis by polymyxin.[19]

Bacterial resistance. No strain resistance with *Eb. typhi, E. coli, K. pneumoniae, S. pullorum* [8, 19, 20]; resistance developed by *S. gallinarum, Past. multocida*.[8]

Factors affecting activity. Peptone, paraaminobenzoic acid, methionine were without effect; lecithin ("Aso-lectin"), soap, "Alconox," lipositol, heating with serum and horse serum lowered activity of Polymyxin D.[7, 25]

PHARMACOLOGY

Toxicity. *Mice*, LD 50 mg/kg [2, 19, 20, 23, 24, 26]:

	Polymyxin A	Polymyxin B	Polymyxin D
intravenous	6.1, 6.9, 9	6.1	11.9, 18
intraperitoneal	13.9, 39	12.1	24.5, 77
subcutaneous	68, 87.5	82.5	160, 230–500

Administration of polymyxin A caused kidney damage, temporary oliguria, albuminuria and tubule degeneration.[24]

Rats: Polymyxin D, 20 mg/kg subcutaneously produced kidney epithelium destruction; not prevented by methionine[26]; partial protection against kidney damage by methionine and by protein hydrolyzate; Polymyxin B did not cause albuminuria.[23]

Rabbits: Polymyxin A intrathecally, average lethal dose 0.6 mg/kg, no vestibular dysfunction noted; subcutaneous administration resulted in albuminuria, disintegration of tubular epithelium and cells of loops of Henle; polymyxin B did not produce albuminuria and only slight kidney changes.[23]

Dogs: Polymyxin D, 2.5 mg/kg intravenous caused death in 20 minutes; 5–10 mg/kg twice a day for 7 days intramuscularly was nontoxic; produced epithelial cells, cellular casts, granular casts in urine, necrosis of tubular epithelium of kidney [26]; only slight kidney pathology with polymyxin B; protection against kidney damage by simultaneous administration of methionine and of protein hydrolyzate [23]; 10 mg of polymyxin D intrathecally produced a temporary paresis.[26]

Clinical: Polymyxin A and D caused albuminuria in man,[23, 28] but not polymyxin B.[23]

Polymyxin A was nontoxic to leucocytes at 1,000 units/ml, non-hemolytic to red cells at 125 units/ml.[20]

Blood levels. In rabbits, polymyxin A 500–2,000 units subcutaneously appeared in blood at 1–3.2 units/ml at 1.5 hours and 0.2 unit/ml at 6 hours. In dogs 5 mg/kg intramuscularly of polymyxin D showed a blood level of 2.5 γ/ml at 210 minutes; 0.25 mg/kg per minute by intravenous drip showed up to 320 γ/ml in blood at 150 minutes after administration was discontinued.[26] In man, 100 units of polymyxin A appeared as 0.2 unit/ml blood level at 6 hours.[20]

Spinal fluid levels. No polymyxin D was detected in the spinal fluid after intramuscular and intravenous administration in dogs[26] and after intramuscular administration in man.[28] In dogs, 1–10 mg intrathecally produced a spinal fluid level of 10–500 γ/ml[26]; clinically a 5 γ/ml level resulted from the intrathecal administration of 2 mg.[28]

Renal threshold. For rabbits, 4 γ/ml of polymyxin A; in mice, only traces appeared in the urine after administration of large doses.[20]

Absorption. Polymyxin A, 4 mg/kg orally in rabbits, could not be detected in the blood or feces; polymyxin-sensitive flora of the intestine showed inhibition.[20]

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POLYPEPTIN

First described by McLeod in 1948[1]; derived from *Bacillus krzemieniewski*. This antibiotic was originally named CIRCULIN, but by agreement of the authors concerned, the name was changed to polypeptin, suggested by its polypeptide constitution.[2]

PRODUCTION

Fermentation. Surface growth on medium containing *dl*-alanine 0.5%, KH_2PO_4 0.5%, magnesium citrate 0.25%, magnesium sulfate 0.05%, glycerol 2.0%, pH 7.2; incubated for 10 days at 37° C, final pH 7.0; yield 300 *E. coli* or 100 hemolytic units/ml. Also by submerged culture in shaker flasks; also incubation at 23° C; also with other amino acids and complex nitrogenous materials as nitrogen sources, and with glucose, sucrose, maltose or lactose as carbon sources.[1]

Isolation. Culture liquor adjusted to pH 2.0, acetone added and ammonium sulfate to saturation; the acetone layer removed and concentrated in vacuo, chloroform added, precipitating crude polypeptin; the precipitate taken up in water, precipitated with ammonium sulfate.[3]

Purification. Crude polypeptin dissolved in 70% ethanol and crystallized; also may be crystallized from 30% methanol, 70% isopropanol, 80% acetone and *M* sodium acetate.[3]

CHEMISTRY

Constitution. Proposed $C_{41}H_{85}O_{15}N_9 \cdot SO_4$, M.W. 1,050.[3]

Properties. Crystal form varies with crystallization solvent; m.p. 235° C; $[\alpha]_{20}^{D} = -93.3°$; ultraviolet absorption bands at 2,520, 2,580 and 2,640; isoelectric point in 0.02*M* citrate buffer at pH 7.0; soluble in water (0.3%), 0.7*M* ammonium acetate (2%), ethylene glycol (5.0%), 70% ethyl alcohol (1.5%) at 25° C.[3]

Reactions. Thermostable in acid or neutral solution; less stable in alkaline solution; no loss of activity by Seitz filtration.[1]

BACTERIOLOGY

Assay. Serial dilution with *E. coli;* crystalline polypeptin 200–300 *E. coli* units/mg. Also by hemolysis of rabbit erythrocytes; crystalline polypeptin 24–27.4 hemolytic units/mg.[1]

Spectrum in vitro. Inhibition concentration (γ/ml)[1]:

Staph. aureus	1.5	*Eb. typhi*	3.0
D. pneumoniae	1.5	*S. paratyphi*	3.0
St. pyogenes	1.5	*S. schottmuelleri*	3.0
E. coli	3.0	*K. pneumoniae*	1.5–3.0
E. communior	1.5	*S. typhimurium*	4.5
Shig. dysenteriae	1.8	*Clos. histolyticum*	1.5
Ps. aeruginosa	10.5	*Clos. fallax*	3.0
B. subtilis	0.75	*Clos. tertium*	7.5
B. mycoides	3.0	*Myco. tuberculosis*	4.5
Clos. perfringens	3.0	*Monilia albicans*	3.0
Trich. rubrum	3.0	*Actino. graminis*	1.5
Epid. floccosum	1.5	*Histop. capsulatum*	1.5
Blas. dermatitidis	1.5	*Micro. audouini*	1.5
Sporo. schenkii	1.5	*Micro. lanosum*	1.5

Factors affecting activity. No effect of 25% serum on activity against *E. coli* or on hemolytic activity.[1]

Mode of action. Bacteriostatic and bactericidal.[1]

PHARMACOLOGY

Toxicity. In mice, LD 50 intraperitoneally was 0.3 mg; somewhat less toxic subcutaneously or in divided doses. Polypeptin was hemolytic for human, rabbit, sheep and mouse red cells.[1]

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POLYPORIN

First described by Bose in 1944 [1]; derived from *Polystictus sanguineus.*[2]

PRODUCTION

Fermentation. Surface growth on medium containing pea-seed infusion, glucose 4%, manganous sulfate and ferrous sulfate; or on Czapek-Dox medium with peptone 1%; initial pH 7.0; incubated at 22–32° C for about 2 weeks; final pH 5.8.[2, 3]

CHEMISTRY

Acidic properties; non-volatile; thermostable; decomposed by heating with alkali or acid; soluble in dry ether, chloroform, ethyl acetate, absolute alcohol, acetone; not inactivated by gastric juice, pepsin, hydrochloric acid.[2, 3]

BACTERIOLOGY

Assay. Cylinder plate with *E. coli, Staph. aureus, St. pyogenes;* also by disk method.[2, 3]

Spectrum in vitro. Active against *Staph. aureus, St. pyogenes, St. viridans, Eb. typhi, S. paratyphi, S. schottmuelleri, E. coli, V. comma, Shig. paradysenteriae.*[3]

Factors affecting activity. Not antagonized by pus, autolyzed tissue, serum or blood.[3]

Spectrum in vivo. Clinically, crude polyporin by local application was effective against infections by *Staph. aureus, St. pyogenes* and *St. viridans.*

By oral administration, favorable results were obtained in preliminary trials on cholera, typhoid and paratyphoid infections. Polyporin neutralized typhoid vaccine.[3]

PHARMACOLOGY

Toxicity. Nontoxic to guinea pigs and rabbits. In man, nonhemolytic and nonpyrogenic on intramuscular administration; not irritating to conjunctiva, superficial open wounds and ulcers.[3]

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PROACTINOMYCIN

First described by Gardner and Chain in 1942[1]; derived from *Nocardia* (*Proactinomyces*) *gardneri*. PROACTINOMYCIN A, B, and C have been described.[4, 5]

PRODUCTION

Fermentation. Surface growth on glucose agar medium for 2 days, glucose broth added, followed by 2 days' additional incubation at 25° C.

Isolation. Culture liquor acidified to pH 5, extracted with ether, ether extract discarded; culture liquor readjusted to pH 10, extracted with ether; ether extract shaken with water at pH 4; aqueous extract concentrated in vacuo and freeze-dried; yield 60 mg/l.[1]

Purification. Countercurrent distribution for separation of proactinomycin A, B, and C.[4]

CHEMISTRY

Amorphous white powder; basic properties; free base soluble in ether, amyl acetate, benzene, carbon tetrachloride; proactinomycin salt soluble in water; precipitated by picric, picrolonic and reinecke acid; stable at pH 2–7; less stable at pH 10.[1]

Constitution.[4]

Proactinomycin

	Proposed	*Calculated*
A	$C_{27}H_{47}O_8N$	C 63.2, H 9.2, N 2.7
B	$C_{28}H_{49}O_8N$	C 63.8, H 9.3, N 2.7
C	$C_{24}H_{41}O_6N$	C 65.6, H 9.3, N 3.2

Found by analysis[4]:

	A	B	C
C	63.2	63.8	65.5
H	9.04	9.4	9.2
N	2.67	2.71	3.08
O	25.09	24.09	22.22
(N)	7.35	8.7	8.5
(C)	18.8	18.04	18.6
OCH_3	1.1	0	0
MW (Rast)	445	513	322

Ultraviolet maximum (in ether solution): proactinomycin A ca. 260 mμ, B ca. 265 mμ, C ca. 300 mμ.[4]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus.*[1,2]

Spectrum in vitro. Inhibition dilution of impure proactinomycin (× 1,000)[1,2]:

St. pyogenes	160–640	*B. anthracis*	256–1,024
St. pneumoniae	200–1,500	*C. diphtheriae*	512–2,048
St. viridans	256	*C. hofmanni*	512–2,048
Staph. aureus	32–640	*V. comma*	6
Neis. meningitidis	500–800	*S. enteriditis*	32–160
S. typhi	<1–2	*Ps. aeruginosa*	<2
E. coli	<1–2	*S. paratyphi*	<2
Proteus sp.	<1	*Shig. dysenteriae*	<2

Fungi, 55 species, showed no inhibition at 1:5,000 or less.[2]

Trypanosoma equiperdum inhibited at 1:10,000; *Leishmania donovani* at 1:10,000; *Endameba histolytica* showed no inhibition at 1:5,000.[2]

Factors affecting activity. No loss of activity by 10% serum [1,2]; some loss by whole blood and lysed red cells.[2]

Resistance. None developed in *St. pyogenes;* no homologous resistance with penicillin by staphylococci.[2]

Synergism. None between proactinomycin and helvolic acid (FUMIGACIN).[2]

Mode of action. At concentrations less than 1:1,000,000, the lag phase of growth was increased; at greater than 1:1,000,000, the lethal effect became evident.[3]

Spectrum in vivo. In mice, partial protection against experimental infections with *St. pyogenes* by oral and intraperitoneal administration of proactinomycin.[2]

PHARMACOLOGY

Toxicity. Lethal doses in mice: 2 mg intravenous, 5–10 mg intraperitoneal, 14 mg oral (by stomach tube). Chronic toxicity with repeated sublethal doses with possible liver damage.[1,2]

At 1:1,000–3,000, killed human leucocytes in one-half to one hour.[1, 2]

Absorption, excretion, blood levels. Proactinomycin was absorbed from the stomach in mice and found in the urine, but no blood levels could be detected in the blood.[2]

Physiology. Slowing of cat heart isolated and in situ, unaffected by atropine; rise of blood pressure; no effect on respiratory rate; inhibited contraction of guinea-pig uterus.[2]

MISCELLANEOUS

As a component of differential culture media, suppressed growth of *Staph. aureus* and *St. pyogenes*, allowed growth of *H. influenzae* and *E. coli.*[2]

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PROACTINOMYCIN A, B, or C see PROACTINOMYCIN

PRODIGIOSIN

Described as a red pigment,[1, 2] derived from *Serratia marcescens* (*Bacillus prodigiosus, Chromobacterium prodigiosum*); found to be antibiotically inactive against 10 representative species of bacteria at 0.005–0.1% concentration.[3]

A nonpigmented, thermostable, water-soluble antibiotic has been described by Lichstein and Van de Sand in 1946,[3] derived from a 3-week culture of *Serratia marcescens*, with bacteriostatic activity against *B. subtilis, C. diphtheriae* and *Staph. aureus.*[3]

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PROTOANEMONIN

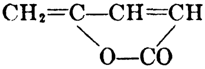
First described and named by Asahina and Fugita in 1922[1] (but cf. the history of anemonin,[5] also cf. ANEMONIN); derived from *Anemone pulsatilla, Ranunculus hirsutus, R. bulbosus.*

PRODUCTION

(A) Steam distillation, saturation of distillate with sodium chloride, extraction with ether, evaporation of ether extract to dryness.[3] (B) Steam distillation, removal of water-insoluble, biologically inactive oil floating on surface of distillate; extraction of distillate with chloroform; evaporation of chloroform extract *in vacuo*, yielding a pale yellow oil.[4] (C) Also prepared by synthesis from β-bromolevulinic acid by way of acetyl-bromolevulinic acid and acetyl-acetacrylic acid lactone.[1,2,7]

CHEMISTRY

Constitution. $C_5H_4O_2$, (dehydro-α-angelica-lactone):



[1,2,3,4,7]

Properties. Pale yellow vesicant oil, stable for many months at 5° C in 1% aqueous solution; polymerizes rapidly in pure state to anemonin and higher polymers.[1,4]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 1,000)[1,2,6]:

Strep. hemolyticus	16–66	*C. xerosis*	6–30
Strep. viridans	33–55	*Myco. tuberc.* hom.	100–330
D. pneumoniae	55	*Myco. tuberc.* bov.	100–250
Staph. aureus	60–150	*Myco. tuberc.* av.	250–450
Staph. albus	66	*B. subtilis*	20–50
M. lysodeikticus	44	*B. anthracis*	20–50
C. diphtheriae	75–100	*Cl. histolyticus*	30–350
C. hofmanni	75–100	*Cl. tetani*	100–120
E. coli	33–83	*Cl. novyi*	30–60
Cl. welchii	30–350	*Cl. oedematiens*	50–100
Cl. sporogenes	30–350		
Sacch. cerevisiae	50–166	*Microsporium audouini*	62–83
Candida albicans	100–200	*Trichophyton purpureum*	83
Cryptococcus neoformans	83–300	*Coccidioides immitis*	125
Trichophyton mentagrophytes	125	*Allescheria boydii*	166
Microsporium canis	125		
Tetrahymena geleii	200	*Trypanosoma gambiense*	200–1,600

No inhibition of coli and staphylococcus bacteriophage, nor of influenza virus in chick embryo.[6]

PHARMACOLOGY

Protoanemonin is toxic to chicken tissue culture at 1:1,000,000.[6]

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PTERYGOSPERMIN

First described by Rao, George and Pandalai in 1946; derived from *Moringa pterygosperma*.

PRODUCTION

Plant root chopped into small pieces, extracted with cold absolute alcohol, active portion adsorbed on activated carbon; eluted with petroleum ether; eluate evaporated in vacuo to an oily residue.

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 1,000):

B. subtilis	75–100	*Eb. typhi*	40–50
Staph. aureus	75–100	*E. coli*	20
Sh. paradysenteriae	40–50	*S. enteriditis*	30–40
Aero. aerogenes	20	*Myco. phlei*	30
S. paratyphi	30–50		

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Rao, R. R., George, M., and Pandalai, K. M., "Pterygospermin: the antibacterial principle of *Moringa pterygosperma*, Gaertn.," *Nature*, **158,** 745 (1946).

PUBERULIC ACID

First described by Birkinshaw and Raistrick in 1932[1]**; derived from** ***Penicillium puberulum, P. aurantio-virens,***[1] ***P. cyclopium-viridicatum, P. johannioli.***[2]

By fermentation, puberulic acid is produced in admixture with PUBERULONIC ACID.[1, 2] The product from *P. aurantio-virens* consisted of approximately equal proportions of the two acids; from *P. cyclopium-viridicatum* the proportion of puberulonic acid predominated. *P. johannioli* yielded almost pure puberulonic acid.[2]

PRODUCTION

Fermentation. *P. aurantio-virens:* surface growth on medium containing g/l glucose 70.0, $NaNO_3$ 15.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.005, incubated at 24° C for 42 days [1]; yield of puberulic acid as crude diacetyl compound and of puberulonic acid as crude acid sodium salt in ratio 1:1.6.[2]

Isolation. Culture filtrate neutralized, activity precipitated with excess nickel sulfate, the precipitate washed, excess HCl added and extracted with ether; ether extract evaporated, residue taken up in methanol and allowed to crystallize; yield 0.33 g/l.[1]

Purification. Diacetyl puberulic acid is formed by heating the crude material with anhydrous sodium acetate and acetic anhydride [1] preferably diluted with glacial acetic acid,[3] at oil bath temperature 140–150° C; the still warm mixture is poured into water and stirred to a deep yellow-brown solution; puberulonic acid removed by crystallization as the acid sodium salt [1]; the mother liquor acidified with 10*N* H_2SO_4 and crude diacetyl puberulic acid separated as a somewhat dark crystalline mass; yield 0.14 g/l; redissolved in hot absolute alcohol, treated with activated carbon and recrystallized twice to yield 0.07 g/l of colorless crystals [1]; diacetyl puberulic acid hydrolyzed by gentle boiling with *N* NaOH in nitrogen atmosphere [1] or with hot aqueous ammonia [3]; hydrolysis mixture cooled and acidified, precipitating free puberulic acid.[1]

CHEMISTRY

Constitution. Proposed $C_8H_6O_6$ by analysis; no methoxyl or aldehyde groups; titration equivalent 95.4, calculated for a dibasic acid.[1, 3]

Properties. From NaOH hydrolysis of diacetyl compound, a cream-colored, microcrystalline powder, not recrystallizable from any solvent; m.p. 316° C (dec.); sublimes in high vacuum at 220° C [1] with 10–15% yield [3]; practically colorless crystalline dibasic acid, m.p. 316–318° C [3]; slightly soluble in water.[1]

From ammonia hydrolysis of diacetyl compound, small colorless prisms, m.p. 300–305° C (dec.).[3]

Reactions. Yellow-brown color with dilute ferric chloride [1]; in culture liquor of *P. puberulum* and *P. aurantio-virens*, brownish red color often accompanied by formation of amorphous black precipitate with ferric chloride.[2]

Derivatives. Diacetyl compound, colorless crystals, m.p. 212° C; monomethyl, dimethyl and diacetyl derivatives, dibasic acids; tetramethyl diacetyl-dimethyl derivatives, and neutral compounds.[1]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus.*[2]

Spectrum in vitro. Inhibition dilution (× 1,000) [2]:

B. anthracis	17–33	*Staph. citreus*	<67
B. coli-commune	<6	*Strep. pyogenes*	6
B. typhi-murium	<6–6	*Strep. viridans*	<6–17
Staph. aureus	<6–<67	*V. cholerae*	<6

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PUBERULONIC ACID

First described by Birkinshaw and Raistrick in 1932 [1]; named by Oxford, Raistrick and Smith in 1942 [2]; derived from *Penicillium puberulum*, *P. aurantio-virens*, *P. cyclopium-viridicatum*, and *P. johannioli* [1, 2]; cf. PUBERULIC ACID.

PRODUCTION

Fermentation. Surface growth on medium containing g/l: (A) glucose 50, $NaNO_3$ 2, KH_2PO_4 1.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01; (B) glucose 75, tartaric acid 4, diammonium tartrate 4, diammonium phosphate 0.6, potassium carbonate 0.6, magnesium carbonate 0.4, ammonium sulfate 0.25, $ZnSO_4 \cdot 7H_2O$ 0.07, $FeSO_4 \cdot 7H_2O$ 0.07; incubated 7–21 days at 24° C.[2]

Isolation. As for PUBERULIC ACID.

Purification. By formation of acid sodium salt (see PUBERULIC ACID, Purification); yield 0.17 g/l; dissolved in methanol, ether added precipitating a dark amorphous precipitate; crystallized from ethyl alcohol; dissolved in hot water, HCl added and free puberulonic acid precipitated by cooling.[1]

CHEMISTRY

Constitution. Proposed $C_8H_4O_6$ by analysis; M.W. 217, 187, calc. 196[1,2]; no aldehyde groups[1,2,3]; sodium salt contains 10.12% sodium.[1]

Properties. Shiny yellow plates, m.p. 296° C (dec.)[1]; bright yellow prisms, m.p. 298° C.[2]

Reactions. With ferric chloride, indistinguishable from PUBERULIC ACID[1,2]; no esters or acetyl derivatives[1,2,3]; orange needles as purified crystalline sodium salt.[1]

BACTERIOLOGY

Assay. See PUBERULIC ACID.

Spectrum in vitro. Inhibition dilution (× 1,000):[2]

B. anthracis	10–17	*Staph. citreus*	17
B. coli-commune	<6	*St. pyogenes*	<6
B. typhi-murium	<6	*St. viridans*	<6–10
Staph. aureus	<6–<17	*V. cholerae*	<6

BIBLIOGRAPHY

See PUBERULIC ACID.

PUCHIIN

First described by Chen, Cheng, Cheng and Tang in 1945; derived from the Chinese water-chestnut, *Eleocharis tuberosa*.

CHEMISTRY

Thermolabile; stable at pH 3–8; not extracted by organic solvents, not adsorbed by carbon or kaolin; activity destroyed by ethanol.

BACTERIOLOGY

Active against *Staph. aureus*, *E. coli*, *Aero. aerogenes;* not against *B. graveolus*.

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PYO COMPOUNDS

The antibiotic activity of the metabolic products of *Pseudomonas aeruginosa* has been studied since the time of Pasteur. (For a brief history, see refs. 1 and 2.) Among the antibiotically active substances isolated (more or less) from *Ps. aeruginosa* cultures, are PYOCYANASE (a commercial product extensively used at the turn of the century for the therapy of diphtheria, grippe and meningitidis), α-OXYPHENAZINE or HEMIPYOCYANIN,[3] PYOCYANIN(E),[4] PYO I (including PYO Ib, PYO Ic), PYO II, PYO III and PYO IV.[1]

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l peptone 10, beef extract 3, sodium chloride 5, glycerol 10, pH 7.5–7.8; incubated at 37° C for 5 weeks; at 7–10 day intervals 10 ml/l of sterile glycerol added and the pellicle broken by shaking.[1] (B) Nutrient broth with 1–2% glycerol; incubated at 37° C for 1–10 weeks.[2] (C) Heart broth with 2% glycerol; incubated for 2 days.[3]

Isolation. *Of Pyocyanin.* Culture liquor extracted with chloroform, followed immediately by extraction of the chloroform layer with dilute HCl.[2,4]

Of α-Oxyphenazine. Culture liquor extracted with chloroform (pyocyanin in chloroform layer rapidly breaks down to α-oxyphenazine); chloroform extract evaporated to dryness, residue taken up in ether.[2]

Culture liquor acidified to pH 3.5 with dilute HCl, centrifuged; the precipitate extracted with hot 95% ethanol, diluted to 80% alcohol, extracted with petroleum ether, petroleum ether extracts discarded; the alcohol layer evaporated and the aqueous layer extracted with ether; the ether solution extracted with dilute sodium bicarbonate, the bicarbonate soluble fraction discarded; the ether solution then extracted with dilute sodium carbonate; PYO II in the carbonate extract, neutralized and extracted into ether; the carbonate-extracted ether solution then extracted with dilute sodium hydroxide to remove residual PYO II; the carbonate-hydroxide-extracted ether solution contains PYO I, III and IV.[1]

Purification. Of PYO II. (A) Crude material dissolved in benzene, passed through an oxalic acid column, the column washed with benzene; the column dissolved in saturated potassium bicarbonate and ether, the ether solution extracted with saturated potassium bicarbonate to remove traces of oxalic acid; the ether solution then extracted with dilute sodium carbonate, the aqueous layer acidified and reextracted into ether; the ether solution washed with water, distilled and the residue crystallized from benzene; the crystals dissolved in alcohol, decolorized with activated carbon and recrystallized from acetone, from methanol and from ethanol;

yield 390 mg from 1,250 liters of culture liquor. (B) Dissolved in alcohol, poured into 10 volumes of *N* sodium hydroxide, chilled and filtered; the filtrate treated with barium hydroxide, filtered, acidified and extracted into ether; crystalline material then prepared as in (A) above.[1]

PYO I, PYO III and PYO IV. Mixture dissolved in chloroform and fractionated on a "Permutit" column by increasing amounts of ethanol in chloroform.[1]

PYO IV. Crude material adsorbed on a "Permutit" column, eluted fractionally with a small amount of ethanol in petroleum ether; crystallized from 1:1 methanol-petroleum ether, from acetone and from methanol.[1]

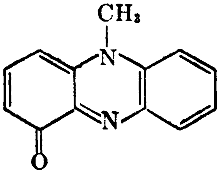
PYO III. Crude material fractionated on a "Permutit" column; crystallized from ethanol-acetone, from methanol, from benzene and from 50% ethanol; decolorized with activated carbon, recrystallized from methanol.[1]

PYO Ic. Crude PYO I dissolved in alcohol-free diethyl ether, washed with *N* potassium hydroxide or 0.1*N* HCl; the ether solution then shaken with *N* HCl and chilled overnight; crystalline hydrochloride removed by filtration, dissolved in ethanol, excess ether added, washed with dilute sodium bicarbonate, with water and distilled; the residue crystallized from 50% aqueous alcohol, decolorized in alcohol with activated carbon, recrystallized from aqueous alcohol.[1]

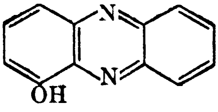
PYO Ib. (A) The *N* potassium hydroxide or 0.1*N* HCl extract (from PYO Ic above) neutralized, the precipitate extracted with ether; the ether extract washed with water, distilled and the residue dissolved in alcohol-free ether; the ether solution extracted with 0.1*N* HCl, the aqueous layer neutralized and reextracted into ether; the ether-acid extraction is repeated exhaustively, the final acid extract decolorized in alcohol with activated carbon and crystallized from acetone. (B) Fractionation on a "Permutit" column in chloroform, eluted with ethanol in petroleum ether, crystallized from 30–40% ethanol and then from acetone.[1]

CHEMISTRY

Constitution[1]



Pyocyanin



α-Oxyphenazine

Pyo Ib $C_{31}H_{40}N_2O_2$ — calc. C 78.77, H 8.53, N 5.93; M.W. 472.6
found — C 78.55, 78.8, H 8.6, 8.7, N 5.7; M.W. 440 (Rast)

Pyo Ic $C_{34}H_{48}N_2O_2$ — calc. C 79.02, H 9.36, N 5.42; M.W. 516.7
found — C 79.4, 79.42, H 9.41, 9.48, N 5.35, 5.3; M.W. 486

Pyo II $C_{34}H_{46}N_2O_4$ — calc. C 74.69, H 8.48, N 5.12; M.W. 546.7
found — C 74.60, 74.49, H 8.52, 8.93, N 5.2, 5.1; M.W. 546
Pyo III $C_{34}H_{44}N_2O_2$ — calc. C 79.64, H 8.65, N 5.46; M.W. 512.7
found — C 79.54, H 8.8, C 5.5; M.W. 502
Pyo IV $C_{16}H_{23}NO_3$ — calc. C 69.28, H 8.36, N 5.05; M.W. 277.4
found — C 69.45, 69.58, H 8.65, N 5.05; M.W. 280, 285

Properties [1]

		M.P. °C
Pyo Ib	colorless crystals	146.2–147
Pyo Ic	colorless crystals	138.8–139.2
Pyo II	light yellow platelets	149 –149.5
Pyo III	colorless crystals	152.8–153.5
Pyo IV	colorless needles	139.5–140 131 –132

Pyo Ib, Ic and III soluble in alcohols, chloroform, benzene, dioxane, acetone and ether; Pyo II soluble in aqueous carbonate, sparingly soluble in cold acetone, benzene, and methanol, insoluble in petroleum ether, water and 5% sodium bicarbonate; characteristic ultraviolet absorption spectra, maxima varying with solvent and acidity or alkalinity.[1]

Reactions. Stable; Pyo Ib and Ic, precipitated from ether solution by oxalic acid, flavianic acid and hydrochloric acid; hydrogenated over platinum oxide in glacial acetic acid. Pyo II, negative with ferric chloride, silver salt, barium salt; reacts with diazomethane; hydrogenated over platinum oxide in glacial acetic acid. Pyo III, hydrogenated over platinum oxide in glacial acetic acid. Pyo IV, hydrogenated over platinum oxide in glacial acetic acid; formed dibenzoate and 2,4-dinitrophenylhydrazone.[1]

Derivatives. Tetrahydro Pyo III, octahydro Pyo Ib, octahydro Pyo Ic, octahydro Pyo II, dodecahydro Pyo III, Pyo IV dibenzoate.[1]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus;* acid-production inhibition with *Staph. aureus* on lactose [1]; oxygen consumption of *Staph. aureus* and *V. comma.*[4] Potency in *Staph. aureus* units per mg: Pyo Ib 160, Pyo Ic 1,280, Pyo II 10,000, Pyo III 300, Pyo IV 120, tetradhydro Pyo III 1,280, octahydro Pyo II 640, octahydro Pyo Ib and Ic 40, dodecahydro Pyo III 40.[1]

Spectrum in vitro. Pyo II units causing "50 per cent acid-production inhibition" (2 units = 1 growth inhibition unit) [1]:

Staph. aureus	1–4	*St. viridans*	256
St. hemolyticus	64	*B. anthracis*	8
C. diphtheriae	4	*B. subtilis*	4
C. hoffmanni	1	*D. pneumoniae*	64
C. xerose	256	*N. catarrhalis*	256

Br. suis	128	*Pr. vulgaris*	256
Br. abortus	64–256	*E. coli*	256
Br. melitensis	256	*Ser. marcescens*	256
Eb. typhi	256	Yeast	256
S. enteriditis	256		

Pyo Ic growth inhibition dilution[1]:

B. subtilis	20	*E. coli*	20
C. xerose	320	*St. hemolyticus*	40
Staph. aureus	320	*Staph. aureus* (penicillin-resistant)	20

Pyocyanin was active against *Staph. aureus*, *E. coli*, *Myco. smegmatis*; α-oxyphenazine against *Staph. aureus*, *Myco. smegmatis* but not against *E. coli*,[2] against numerous gram-positive and gram-negative bacteria.[4, 6, 7]

Mode of action. Bacteriostatic.[1]

Factors affecting activity. Activity lowered by horse serum; slight enhancement by riboflavin.[1]

Spectrum in vivo. In mice, Pyo II administered intraperitoneally did not protect against experimental infection with *D. pneumoniae*; in guinea pigs, subcutaneous administration did not protect against *Myco. tuberculosis hominis*.[5]

PHARMACOLOGY

Toxicity. Pyocyanin: MLD for mice about 2 mg intraperitoneally; inhibited growth of fibroblasts in tissue culture at 0.2–1.0 mg/ml.[4]

α-Oxyphenazine: 10 mg intraperitoneally not lethal to mice.[4]

Pyo II: in mice, MLD intraperitoneally about 0.75 mg; when dissolved in mouse fat, 6 out of 10 died with 2.5 mg; no hemolysis of washed rabbit erythrocytes.[5]

Crude Pyo extract in benne oil nontoxic to mice at 5.5 mg subcutaneously, at 10 mg in four doses during 48 hours intravenously; up to 100 mg orally was nontoxic.

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PYOCYANASE See PYO COMPOUNDS

PYOCYANIN(E) See PYO COMPOUNDS

PYOLIPIC ACID

First described by Bergstrom, Theorell and Davide in 1946; derived from *Pseudomonas aeruginosa* (*pyocyanea*).

PRODUCTION

Fermentation. On synthetic medium; yield 1.0 g/l.

Isolation. Bacterial cells extracted with "suitable" solvent; fractionated with buffer extractions.

CHEMISTRY

Constitution. Proposed $C_{11}H_{22}O_3$; contains *dl*-β-hydroxydecanoic acid and a carbohydrate; neutralization equivalent 500.

Properties. Colorless, odorless, viscous oil soluble in most fat solvents; $[\alpha]_D^{20} = -18.9°$; lead salt insoluble in water.

BACTERIOLOGY

Assay. Manometric, oxygen consumption with *M. tuberculosis*.

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QUERCETIN

Antibacterial activity first observed by Naghski, Copley and Couch in 1947.

CHEMISTRY

Constitution. 5,7,3′,4′-tetrahydroxy-flavonol.

BACTERIOLOGY

Spectrum in vitro. At concentration 0.075–0.10 mg/ml completely inhibited *Staph. aureus*, *Staph. albus*, *Aero. polymyxa*, *Br. abortus;* in concentration 0.15 mg/ml partially inhibited a strain of a group D and a group A streptococcus, and *E. coli*, *Salm. oranienburg*, *Proteus* sp., *Ps. aeruginosa*, *Ps. angulata*, *Ps. tobaci*, *Aero. aerogenes*.

At 0.15 mg/ml partially inhibited *Mucor racemosus* but not *A. fumigatus*, *A. niger*, *F. oxysporum* var. *lycopersici*, *P. notatum* and *Actinomyces fradii*.

Mode of action. Bactericidally active against *S. aureus* in concentrations 0.10–0.15 mg/ml; rate of killing proportional to concentration.

Factors affecting activity. Activity lost in presence of serum and iron but not of cystaine.

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RAPHANIN

First described by Ivanovics and Horvath in 1947[1]; derived from the seeds of *Raphanus sativus.*

Raphanin occurs in the radish seed as an antibiotically inactive precursor, which is converted by a concomitant enzyme into the active product by incubation in aqueous solution. By prolonged incubation, raphanin is further enzyme-converted into an antibiotically inactive product.[2]

PRODUCTION

Isolation. Finely ground radish seeds extracted with water, aqueous filtrate adjusted to pH 5, lead acetate added, precipitate discarded, excess lead removed with Na_2HPO_4; yield 7,370 arbitrary units from 2 kg of seed.[2]

Purification. Aqueous solution extracted with butyl acetate, solvent removed in vacuo, brown oily residue shaken with pH 7.2 phosphate buffer; water-soluble layer extracted with chloroform, chloroform extract washed with phosphate buffer, then passed through an alumina column; the percolate vacuum-evaporated to a yellow syrup and distilled in vacuo, yielding as distillate pure raphanin; yield 7.5 g/2 kg of seed.[2]

CHEMISTRY

Constitution. By analysis[2]:

	C	H	N	S
$C_{17}H_{26}N_3O_3S_5$	42.48	5.45	8.74	33.35
$C_{17}H_{26}N_3O_4S_5$	42.11	5.28	8.58	33.03
Found	41.50	5.50	8.91	33.64

Properties. Yellowish liquid with radish-like odor, b.p. 135° C at 0.06 mm Hg; soluble in water with neutral reaction; soluble in ethanol, butanol, ethyl acetate, butyl acetate, amyl acetate, chloroform; moderately soluble in ether, petroleum ether; $[\alpha]_D^{20} = -141°$ in ethanol; adsorbed on activated carbon but no elution; no adsorption on tricalcium phosphate, kaolin; alkali-labile, more stable in neutral or slightly acid solution.[1, 2]

Reactions. No color with ferric chloride, nitroprusside; after HCl and Zn dust treatment, purple color with nitroprusside; black precipitate with Ag or Pb salts; crystalline decomposition product by evaporation of butyl acetate solution or by treatment with barium hydroxide; inactivated by H_2S, Pb salts.[1, 2]

BACTERIOLOGY

Spectrum in vitro. Inhibition dilution (× 1,000):[1, 2]

Staph. aureus	1–50
E. coli	1–125
S. schottmuelleri	8
Ps. aeruginosa	4–16
S. typhi	4–16

Also active against *Shig. dysenteriae*, *B. anthracis* (avir.), *B. subtilis*, *B. prodigiosus*.[1, 2]

PHARMACOLOGY

In mice, intravenously and subcutaneously, 7–10 mg was lethal; in guinea pigs, 50 mg intracardially was lethal. Raphanin at 1:20,000 dilution inhibited growth of tissue culture of fibroblasts from rabbit testis[2]; isolated frog heart slowed and stopped by 1:800 raphanin.[1]

MISCELLANEOUS

Raphanin inhibited germination of seeds of several plant species, including those of the radish.[1, 2]

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ROCCELLIC ACID

Roccellic acid, α-methyl-α′-*n*-dodecylsuccinic acid, derived from the lichen, *Lecanora sordida*, was only feebly inhibitory to *Myco. phlei* and *Myco. tuberculosis bovis*. By treatment of the anhydride with methyl alcohol and neutralization with dilute alkali, a mixture is obtained of the isomeric monomethyl esters: α-ethyl-α′-dodecylsuccinic monomethyl ester and α-α′-di-*n*-heptylsuccinic monomethyl ester. These derivatives inhibited *Myco. tuberculosis bovis* at 1:300,000–400,000, *Staph. aureus* at 1:20,000; *C. diphtheriae mitis* at 1:40,000; *E. coli* and *Aero. aerogenes* were inhibited at $<$1:1,000.

BIBLIOGRAPHY

(1) Barry, V. C., and McNally, P. A., "Inhibitory action of dialkyl succinic acid derivatives on the growth *in vitro* of acid-fast bacteria," *Nature*, **156**, 48 (1945).

SAMBUCININ See LATERITIIN GROUP

SIMPLEXIN

First observed by Cordon and Haenseler in 1939 [1]; named by Foster and Woodruff in 1946 [2]; derived from *Bacillus simplex*.

PRODUCTION

Fermentation. On medium containing corn steep liquor.[1]

Isolation. Activity of culture filtrate adsorbed on activated carbon, eluted with methanol; eluate evaporated in vacuo to dryness, residue taken up in water.[2]

CHEMISTRY

Properties. Acid-stable, alkali-labile; insoluble in chloroform, ether, benzene, ethyl acetate, *n*-butanol; soluble in methanol, 95% alcohol.[3, 4]

BACTERIOLOGY

Spectrum in vitro. Inhibition of *Rhizoctonia solani*.[1]

Spectrum in vivo. In mice, no protection against experimental infections with *Staph. aureus* and *D. pneumoniae*.[2]

PHARMACOLOGY

Toxicity. In mice, intraperitoneally, 20 mg was the highest tolerated dose.[2]

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SPINULOSIN

First described by Birkinshaw and Raistrick in 1931 [1]; named by Anslow and Raistrick in 1938 [2]; derived from *Penicillium spinulosum*,[1] *P. cinarescens*,[5] *Aspergillus fumigatus* [4] and by synthesis.[2, 3]

PRODUCTION

Fermentation. Surface growth: (A) With *P. spinulosum* on Czapek-Dox medium with added iron incubated at 22° C for 14 days.[1]

(B) With *A. fumigatus* on medium containing g/l glucose 50, tartaric acid 2.7, ammonium tartrate 2.7, ammonium monophosphate 0.4, potassium carbonate 0.4, magnesium carbonate 0.27, ammonium sulfate 0.16, zinc sulfate heptahydrate 0.05, iron sulfate heptahydrate 0.05; incubated at 24° C for 25–26 days, final pH 5.86.[4]

(C) With *P. cinarescens* on Czapek-Dox medium incubated 12–15 days, final pH 3.8–3.9; yield 80 *Staph. aureus* dilution units per ml.[5]

Isolation. (A) Culture filtrate concentrated in vacuo, refluxed with sulfuric acid for several hours, cooled and extracted with ether; the ether solution evaporated precipitating some spinulosin; the mother liquor dissolved in water and extracted with toluene; the toluene extract concentrated in vacuo to crystallize out the residual spinulosin.[1]

(B) Acidified culture filtrate extracted with ether, ether solution washed with water, dried over anhydrous magnesium sulfate and concentrated; the ether concentrate extracted with pH 7.0 buffer; the buffer extract acidified and reextracted with ether, the ether extract dried and evaporated.[4]

(C) Culture filtrate extracted with chloroform; the chloroform extract washed with water and evaporated in vacuo to dryness; the oily residue dissolved in ether, filtered and extracted with saturated aqueous sodium bicarbonate; the aqueous extract acidified, extracted with chloroform, the chloroform extract evaporated.[5]

Purification. Sublimation in vacuo followed by recrystallization from toluene.[1, 4]

Synthesis. (A) Conversion of FUMIGATIN to the tetraacetate, hydrolysis to tetrahydroxymethoxytoluene and oxidation to spinulosin.[2]

(B) From toluquinone → 4-methoxytoluquinone → 2,3,6-triacetoxy-4-methoxy-toluene → 2,3,6-trihydroxy-4,4-methoxytoluene → 6-hydroxy-4-methoxytoluquinone → 4-methoxy-3,6-di(methylamino)-2,5-toluquinone → spinulosin; yield about 30% from 4-methoxytoluquinone.[3]

CHEMISTRY

Constitution. 6-hydroxyfumigatin (3,6-dihydroxy-4-methoxy-2,5-toluquinone); $C_8H_8O_5$; calculated C 52.16, H 4.38; M.W. 184; found C 52.14, 52.28, H 4.29, 4.47; M.W. 184 (Rast in camphor).[1, 2, 3, 4]

Properties. Black crystals with metallic luster; brownish plates under microscope; aromatic odor characteristic of quinones; m.p. 201° C after sublimation,[2, 3] 202–203.5° C [1]; slightly soluble in cold water, more soluble in hot [1, 2]; polarographic properties.[11]

Reactions. Intense blue color with concentrated sulfuric acid; blue-purple color with normal sodium hydroxide; intense brown color in alcohol with ferric chloride; decolorizes cold acidified permanganate; reduced by zinc and HCl to a colorless compound which gives a blue-green color with ferric chloride; color change with pH, below pH 2.8 light buff, at pH 3.1 pink-violet, at pH 5.5 deep purple, above pH 8.9 almost colorless; thermostable at pH 2.0–9.5, less stable above pH 9.5.[1, 2, 3, 7, 11]

BACTERIOLOGY

Assay. Cylinder plate with *Staph. aureus*.[7]

Spectrum in vitro. Inhibition concentration (γ/ml) [8, 10]:

Staph. aureus	63–170	*E. coli*	100–250
Staph. albus	170	*Ps. aeruginosa*	500
B. mycoides	125	*K. pneumoniae*	250
B. subtilis	125	*S. typhimurium*	170
B. anthracis	170	*V. comma*	170
Myco. phlei	250	*Myco. smegmatis*	500
Photo. fischeri	16		

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(2) Anslow, W. K., and Raistrick, H., "XCI. Studies in the biochemistry of microorganisms. LVII. Fumigatin (3-hydroxy-4-methoxy-2,5-toluquinone) and spinulosin (3,6-dihydroxy-4-methoxy-2,5-toluquinone), metabolic products respectively of *Aspergillus fumigatus* Fresenius and *Penicillium spinulosum* Thom," *Biochem. J.*, **32,** 687 (1938).

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STREPTIN

First described by Woodruff and Foster in 1946; derived from *Streptomyces* sp.

Production: By procedure used for streptothricin.

Bacterial spectrum: Similar to that of streptothricin, but more active against staphylococci and micrococci.

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Woodruff, H. B., and Foster, J. W., "Streptin, an antibiotic from a species of streptomyces," *J. Bact.*, **52**, 502 (1946).

STREPTOCIN

First observed by Schatz and Waksman in 1944 [1]; isolated in admixture with other antibiotics by Waksman, Schatz and Reilly in 1946 [2]; isolated in crystalline form and named by Waksman, Harris, Kupferberg, Singher and Styles in 1949 [3]; derived from *Streptomyces griseus*.

PRODUCTION

Fermentation. Surface or submerged growth on glucose-peptone-meat extract-NaCl-tap water; medium maximum yield in surface growth on fifth day, 27.0 *Trichomonas vaginalis* units/ml (76% in mycelium, 24% in culture filtrate); yield in submerged growth on second day, 9.0 *T. vaginalis* units/ml (66% in mycelium, 33% in culture filtrate).[3]

Isolation. Mycelium extracted in Soxhlet with aqueous ether, the ether layer evaporated in vacuo to a brown oil, extracted with absolute ethanol; ethanol solution distilled in vacuo to a dry yellow solid; the residue extracted with petroleum ether, the petroleum ether extract evaporated to dryness. (The petroleum ether-insoluble fraction contained material of similar properties as indicated by chromatography.)

Purification. Chromatography in petroleum ether solution on an alumina column, developed with benzene, acetone, ethanol, methanol; eluted with 50–50 methanol-ethanol, then with 1% glacial acetic acid in methanol; or by sublimation at 100° C in vacuo at 10^{-5} mm.[3]

CHEMISTRY

Properties. Needle-like crystals in rosettes; crystallizable from 1% glacial acetic acid in methanol or from acetone, but not from amyl acetate; crystalline structure lost on standing; soluble in water, methanol, ethanol; insoluble in chloroform; forms gel in amyl acetate; may be sublimed; no characteristic ultraviolet spectrum; thermostable; stable at pH 2.0–11.0.[3]

BACTERIOLOGY

Assay. Serial dilution, using added streptomycin and penicillin to prevent contamination, with *Trichomonas vaginalis* as test organism; sublimed streptocin, 153 *T. vaginalis* units/mg.[3]

Spectrum in vitro. Inhibition concentration (units/mg):[3]

E. coli	<3	*Myco. ranae*	<8
B. mycoides	3	*Mycobacterium*	13
Staph. aureus	3	*Myco. avium*	8
B. subtilis	5		

Inhibition of *T. vaginalis* at 1:29,000; partial inhibition of *T. foetus* Br. at 50 γ/ml; no effect on *T. gallinae* at 50 γ/ml.[3]

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STREPTOLIN

First described by Rivett and Peterson in 1947; derived from *Streptomyces* sp.

PRODUCTION

Fermentation. Submerged culture in shaker flasks on medium containing g/l (A) soybean meal 20, "Curbay BG" 5, glucose 20, NaCl 10, $CaCO_3$ 1.0; incubated at 25° C for 72 hours; yield 35,000 *E. coli* units/ml. (B) Ammonium nitrate 0.5, sodium nitrate 5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.05, glucose 10, $CaCO_3$ 1.0, K_2HPO_4 6, pH 8; incubated at 25° C for 77 hours; yield 10,400 units/ml.

Submerged culture in 30-liter fermenter on medium containing (A) g/l soybean meal 10, corn-steep liquor solids 10, glucose 10, NaCl 5, $CaCO_3$ 1.0; incubated at 25° C for 70 hours, air ratio volume per minute to medium volume 0.25, at RPM 195; yield 48,600 units/ml. (B) Synthetic medium as for shaker flasks (see above); incubated at 25° C for 93 hours; yield 8,900 units/ml.

Isolation. Culture liquor adjusted to pH 2.5 with sulfuric acid, "Celite 545" added, and filtered; the filtrate adjusted to pH 7.9 and filtered; the activity of the filtrate adsorbed on "Filter-Cel" and eluted with aqueous pyridine hydrochloride at pH 1.5; the activity of the eluate adsorbed on activated carbon at pH 2.3, and eluted with acid aqueous ethanol; the eluate concentrated *in vacuo*, adjusted to pH 7.6 with sodium hydroxide and pyridine removed by distillation; sodium chloride removed by precipitation with methanol; crude streptolin precipitated with ethyl ether; recovery 60–70%.

Purification. Precipitated as the helianthate and converted to the hydrochloride.

CHEMISTRY

Constitution. Streptolin helianthate: N 12.69, helianthine 63.6.

Properties. Optical rotation $[\alpha]_D^{28} = -22°$ ($c = 1.8$), $= -25°$ ($c = 2.9$); diffuses slowly through agar. Helianthate m.p. 207–211° C (dec.).

Reactions. Maximum thermal stability at pH 3–3.5; maltol negative.

BACTERIOLOGY

Assay. Turbidimetric with *E. coli*. Streptolin hydrochloride 33,000–34,000 units/mg; streptolin helianthate 15,200–15,400 units/mg.

Spectrum in vitro. Inhibition concentration (units/ml):

Aero. aerogenes	3.3	*B. mycoides*	10.0
Aero. polymyxa	1.0	*E. coli*	1.0
B. cereus	3.3	*Pr. vulgaris*	3.3
B. fusiformis	0.1	*Ps. aeruginosa*	10.0
B. megatherium	0.3	*Ps. fluorescens*	33.0

PHARMACOLOGY

In mice, intravenously, 8–10 mg/kg was lethal.

BIBLIOGRAPHY

(1) Rivett, R. W., and Peterson, W. H., "Streptolin, a new antibiotic from a species of *Streptomyces*," *J. Am. Chem. Soc.*, **69**, 3,006 (1947).

STREPTOMYCIN

(The following is a summary in extremely abbreviated form of the basic information relating to streptomycin; see Introduction, page 4.)

First described by Schatz, Bugie and Waksman [*Proc. Soc. Exper. Biol. Med.*, **55**, 66 (1944)], derived from *Streptomyces griseus*.

Several types of streptomycin have been described: STREPTOMYCIN, DIHYDROSTREPTOMYCIN, MANNOSIDOSTREPTOMYCIN or STREPTOMYCIN B and DIHYDROMANNOSIDOSTREPTOMYCIN. STREPTOMYCIN II is derived from *Streptomyces bikiniensis*.

PRODUCTION

Fermentation. Surface and submerged growth on media containing as nitrogen sources, meat extract, peptone, corn-steep liquor, soybean meal, distillers' wastes, or ammonium salts; as carbon source, glucose or other carbohydrate; with sodium chloride, calcium carbonate, phosphates and trace elements; incubated at 25–28° C for 7–10 days in surface culture, 2–3 days in submerged culture; yields 70 γ/ml of free base in surface culture; up to 1,000 γ/ml of free base have been reported in submerged culture.

Isolation. Culture filtrate activity adsorbed on activated carbon; eluted with acid alcohol.

Purification. Chromatography; precipitation as the picrate, reineckate, helianthate, phosphotungstate, molybdate or *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonate; as the crystalline double salt with calcium chloride.

CHEMISTRY

Constitution. Proposed $C_{21}H_{37-39}N_7O_{12}$; a hydroxylated base, streptidine, with a glycoside linkage to an amino-saccharide, streptobiosamine. Streptidine has been identified as a guanidine derivative; streptobiosamine contained streptose and N-methyl-1-glucosamine.

Properties. Marked basic properties; soluble in water, less soluble in lower alcohols, insoluble in other organic solvents.

Reactions. Fairly stable in solution at room temperature, and in dilute acids and alkalies; maltol produced by alkali hydrolysis.

BACTERIOLOGY

Assay. Cylinder-plate with *E. coli* or *B. subtilis;* maltol determination. One *E. coli* unit is equivalent to 1.0 gamma of pure free streptomycin base.

Spectrum in vitro. Active against a large number of gram-positive and gram-negative organisms, including staphylococci, streptococci, bacilli, corynebacteria, the colon-typhoid group, *Brucella*, *Hemophilus* and *Mycobacteria* over a range of 0.1–2.0 γ/ml. Little to no activity against yeasts, fungi, viruses, rickettsiae, anaerobic bacteria and protozoa, and against resistant strains of susceptible organisms.

Factors affecting activity. Antagonized by cysteine, hydroxylamine, glucose, sulfhydryl and ketone compounds, and by anaerobiosis.

Synergism. Streptomycin activity against tuberculosis enhanced by promin and by paraaminobenzoic acid.

volume 0.25, at RPM 195; yield 48,600 units/ml. (B) Synthetic medium as for shaker flasks (see above); incubated at 25° C for 93 hours; yield 8,900 units/ml.

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Factors affecting activity. Antagonized by cysteine, hydroxylamine, glucose, sulfhydryl and ketone compounds, and by anaerobiosis.

Synergism. Streptomycin activity against tuberculosis enhanced by promin and by paraaminobenzoic acid.

Resistance. Exposure to sub-bacteriostatic concentrations almost invariably results in acquired resistance by susceptible organisms; naturally resistant strains frequently encountered. Dependent strains (utilizing streptomycin as an essential nutrient) have been described.

Mode of action. Bacteriostatic; bactericidal at high concentration.

Spectrum in vivo. Effective by parenteral administration in infection with susceptible organisms in animals and clinically; effectiveness lessened when acquired resistance occurred. Streptomycin is the only chemotherapeutic agent used successfully in the treatment of acute tuberculosis, particularly tubercular meningitis and septicemia; against pulmonary and chronic forms of tuberculosis, the action of streptomycin was largely suppressive.

PHARMACOLOGY

Toxicity. The toxicity of streptomycin is related partially to the degree and type of impurity associated with the streptomycin. In mice, an LD 50 of 225 mg/kg and a minimal lethal dose of 0.75–1.5 g/kg, administered parenterally, have been reported; liver and kidney pathology has been observed in laboratory animals but not in man.

Clinically, neurotoxic reactions involving the labyrinth of the inner ear have been encountered, following prolonged administration.

Blood levels. Therapeutic blood levels are maintained in the blood for 2–6 hours after administration of 0.5 g parenterally.

Spinal fluid levels. Small amounts of streptomycin were detected in the spinal fluid following parenteral administration.

Fetal blood levels. Significant blood levels found in the placenta following administration into maternal blood stream.

Excretion. Streptomycin is rapidly excreted, about one-half of the administered dose being recovered in the urine.

Absorption. Administered orally, only a trace of streptomycin appears in the blood stream; over half the ingested dose can be accounted for in the feces.

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STREPTOTHRICIN

First described by Waksman, Woodruff and Horning in 1941 [1]; derived from *Streptomyces lavendulae* [2] and from *Streptomyces* sp.[3, 4]

PRODUCTION

Fermentation. With *S. lavendulae* by surface growth. (A) On medium containing g/l glucose or starch 10, "Tryptone," glycine or glutamic acid 5, K_2HPO_4 0.5–2.0, NaCl 0.5–2.0, $FeSO_4$ 0.01–0.1 with agar 2.5 or cotton to maintain a surface pellicle for higher yields; incubated at 20–28° C for 7–12 days; yield up to 200 *B. subtilis* or 75 *E. coli* units per ml.[5, 6, 7]

(B) On medium containing g/l glycerine 5, "Tryptone" 5, K_2HPO_4 5, $FeSO_4 \cdot 7H_2O$ 0.01, agar 2; incubated at 28° C for 5–8 days; final pH 8.0–8.4; yield 400–500 units per ml.[8]

(C) On media containing alanine, aspartic acid, asparagine, and yeast extract.[5, 7]

With *S. lavendulae* by submerged growth. (A) In shaker flasks incubated at 30° C on medium containing dextrose 2%, corn-steep liquor 3%; yield 70 *E. coli* units per ml in 7 days; on medium containing dextrose 1%, soybean meal 0.6%, yield 100 *E. coli* units per ml in 5 days.[7]

(B) In shaker flasks on medium containing "Tryptone" 0.5%, starch 1.0%; incubated 2–5 days; yield up to 1,000 *B. subtilis* or 150 *E. coli* units per ml.[6]

(C) In aerated tanks on medium containing dextrose, starch, sucrose, maltose or glycerol 0.5–2%, corn-steep liquor, soybean meal, "Tryptone," or an amino acid 0.1–5.0%, K_2HPO_4, $MgSO_4 \cdot 7H_2O$ and NaCl; on medium containing dextrose 1%, soybean meal 0.6%, K_2HPO_4 0.2%, NaCl 0.2; incubated for 2–5 days; yield 50–100 units per ml.[9]

Isolation. Neutralized culture filtrate activity adsorbed on activated carbon. (A) Activity eluted with dilute mineral acid, eluate neutralized and evaporated in vacuo to dryness; residue extracted with absolute alcohol, the extract filtered and evaporated to dryness.[5]

(B) Activity eluted with acid alcohol, the eluate neutralized and ether added; the aqueous phase separated and concentrated in vacuo.[6]

(C) Carbon cake washed with ethanol and dried in vacuo; activity eluted with $0.8N$ formic acid in 50% aqueous methanol; eluate concentrated in vacuo, methanol and acetone added, precipitating streptothricin formate; precipitate washed with ether and dried in vacuo; yield 30–60%.[10]

Neutralized culture filtrate activity adsorbed on cation exchanger. (A) After washing cation exchanger with water and acetone, and drying until acetone-free, activity eluted with saturated NaCl solution; eluate evaporated to dryness in vacuo, residue extracted with boiling methanol;

methanol extract filtered and chilled, ether added precipitating streptothricin; the precipitate washed with absolute ethanol, evaporated to dryness, the residue taken up in water; the aqueous solution treated with activated carbon and freeze-dried; yield 70–80% of activity in culture filtrate.[8]

(B) Activity eluted with propylamine or ethylenediamine hydrochloride with 90–100% recovery; also with saturated KCl solution, HCl, sodium bicarbonate, ammonium chloride, guanidine hydrochloride or piperidine hydrochloride with 10–80% recovery.[8]

Purification. (A) Streptothricin precipitated from eluate with phosphotungstic acid, free base liberated by addition of alkali; free base converted into the picrate, fractionated chromatographically and picric acid removed; precipitated with Reinecke's salt as crystalline streptothricin reineckate; fractionally recrystallized.[11]

(B) Crude streptothricin dissolved in hot water, picric acid added, the solution cooled to about 5° C, the precipitate of streptothricin picrate and free picric acid dissolved in warm acid methanol, precipitated with ether and the precipitate dried in vacuo; yield 40–80%.[10]

(C) Also purification by precipitation as the helianthate,[12] the formate, phosphotungstate, picrolonate and flavianate[10]; the sulfate formed from the reineckate by treatment with silver sulfate[11, 12]; the hydrochloride formed from the helianthate by methanolic HCl, removal of the helianthic acid and precipitation with ether.[10, 12]

CHEMISTRY

Constitution. For free base, proposed $C_{13}H_{25}O_7N_5$ from di-reineckate, $C_{21}H_{39}O_7N_{17}S_8Cr_2$ found by analysis[11]; found C 50.36, 50.40, H 5.14, 5.44, N 16.86, 16.84, from belianthate.[10]

Streptothricin hydrochloride, found by analysis: C 36.60, 36.42, H 6.04, 6.20, N 13.42, Cl 14.80, no ash, sulfur or phosphorus[10]; contains at least 5 nitrogen atoms, two of which are basic and salt-forming; no O-methyl, N-methyl or hydrolyzable acetyl groups; may contain 3–4 OH groups.[11]

Streptothricin sulfate, C:N ratio about 13:5.[11]

Dibenzoyl derivative, found $C_{13}H_{25}H_{25}O_7N_5$.[11]

Properties. Streptothricin hydrochloride: white powder; soluble in water and dilute mineral acids; insoluble in ether, petroleum ether and chloroform.

Streptothricin sulfate: hygroscopic white powder amorphous and crystalline.[8, 10, 11]

Streptothricin reineckate: fine platelets in clusters; sintered at 184° C, decomposed at 192–194° C (corr.).[11]

Streptothricin helianthate: crystalline; m.p. 220–226° C (dec.),[12] 225–230° C (dec.) [10]; insoluble in aqueous methanol, almost insoluble in water; solvent of crystallization removed by 100° C in vacuo.[12]

Reactions. Thermolabile in culture filtrate and when alcohol-precipitated; thermostable when purified; destroyed by concentrated acids; precipitated from "Tryptone" medium by protein precipitants, but not after isolation; not digested by proteolytic enzymes.[1, 5, 8]

Reduces Tollen's reagent, neutral permanganate and boiling Fehling; positive biuret, ninhydrin; negative ferric chloride, nitroprusside, Molisch, Sakaguchi and Schiff.[11]

Derivatives. No crystalline derivatives with aliphatic and aromatic acyl chlorides; with benzoyl chloride in aqueous bicarbonate, a dibenzoyl derivative; in pyridine a penta- or hexabenzoyl derivative of pyridine [11]; crystalline salt with *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonic acid [12]; sparingly soluble salts with flavianic, azobenzenesulfonic, nitranilic and rhodanilic acids.[8]

BACTERIOLOGY

Assay. Agar plate dilution with *E. coli* and with *B. subtilis* [6, 13, 14]; serial dilution with *E. coli* [5, 7, 13, 15, 16, 17, 19, 20]; in the presence of streptomycin [18]; cylinder plate with *B. subtilis* [7, 20]; ratio of activity against *E. coli* and *B. subtilis* 1:5 to 1:10.[6] Potency of crystalline reineckate 256 units/mg [11]; of amorphous sulfate 500–530 units/mg [11]; of crystalline sulfate 300 units/mg [12]; of crystalline helianthate 350 *B. subtilis* units/mg,[12] 340 units/mg [10]; of crystalline salt with *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonic acid 300 units/mg [12]; of hydrochloride 800–830 units/mg.[10, 12]

Spectrum in vitro. Inhibition concentration in *E. coli* units/ml [13, 15, 21]:

St. hemolyticus	32, 256	*B. mycoides*	>16, 1,024
St. viridans	>16, 1,024	*B. subtilis*	0.5, 32
St. lactis	4, 1,024	*Eb. typhi*	0.25, 4
Staph. aureus	1, 128	*S. aertryke*	2, 16
D. pneumoniae	4, 32	*S. enteriditis*	0.5, 64
P. leptiseptica	0.5, 32	*S. schottmuelleri*	2, 16
Pr. vulgaris	8, 512	*Sh. dysenteriae*	32
Ps. aeruginosa	>16, 256	*Sh. sonnei*	1, 128
N. intracellularis	256	*Sarcina lutea*	256
E. coli	0.5, 16	*Aero. aerogenes*	1, 256

Cl. welchii	>104, >855
Cl. tetani	>104, >1,080
Cl. sordelli	>104, >1,080
Cl. septique	52, 540
Cl. novyi	270

With crude streptothricin, inhibition concentration in dilution units[20]:

B. subtilis	30,000	*E. coli*	3,000
B. mycoides	<100	*Sh. gallinarum*	5,000
Staph. aureus	2,000	*S. pullorum*	5,000
Myco. phlei	5,000	*Ser. marcescens*	200
Myco. tuberc. hom.	100	*Ps. aeruginosa*	50

With purified streptothricin (units activity × 1,000)[6]:

E. coli	100	*Sh. paradysenteriae*	50
Aero. aerogenes	30	*B. subtilis*	750
Ps. fluorescens	<10	*B. mycoides*	<10
Sh. gallinarum	300	*B. cereus*	<10
Sh. dysenteriae	30–100	*Staph. aureus*	200
		Sarcina lutea	100

Activity against fungi, inhibition concentration in *E. coli* units/ml[21]:

Asp. niger	250	*Micro. canis*	1,000
Pen. chrysogenum	250	*Sab. schenki*	250
Cryp. neoformans	250	*Trich. gypseum*	500
Ep. inguineae	1,000	*Trich. interdigitale*	1,000

Bacteriostatic activity against *Myco. tuberculosis*, *Myco. leprae*, *Myco. smegmatis*, 0.1–1.0 unit/ml, bactericidal at 4.0–5.0 units/ml[22]; against *Eb. muriseptica*, 6.6 *E. coli* units/ml[23]; inhibition of yeast form of *Blastomyces dermatitidis* at 10 units/ml[41]; of *Mal. mallei* at 0.1–1.0 unit/ml; active against *B. abortus*,[26, 27] *B. megatherium*, *H. influenzae*, *Ach. agile*, *Ach. vinelandii*, *Micro. lysodeikticus*; less active against *B. polymyxa*, *Past. pseudotuberculosis*, *S. arbotivoequina*, *S. typhimurium*, *H. suis*, *Ach. chroococcum*, *Ach. indicum*, *Myco. phlei*, *Ac. butylicum*, *Lact. casei*, *Act. albus*, *Act. lavendulae*.[5, 27, 28, 29, 30, 32]

Factors affecting activity. Activity not reduced by cysteine against *B. subtilis*[37]; not markedly affected by number of organisms, by blood, serum, peptone, B-complex vitamins[13]; influenced by pH, inorganic salts.[16]

Mode of action. Bacteriostatic and bactericidal.[1, 5, 26, 27, 28, 29]

Spectrum in vivo. In mice, experimentally infected with *S. schottmuelleri*,

intravenous therapy:	100 units, single dose	94% survival
	50 units/day for 3 days	100% survival
intraperitoneal therapy:	50 units, single dose	92.5% survival
subcutaneous therapy:	200 units, single dose	100% survival
oral therapy:	3,000 units, single dose	76% survival

With *E. coli*, *S. aertrycke* or *Eb. typhi*, 50% protection by 100 units; with *St. hemolyticus*, protection by 1,600 units; with *Pr. vulgaris*, *Ps. aeruginosa*, prolongation of life, but little protection; no protection against *D. pneumoniae*.[13] With streptothricin (5–300 units/mg), 2,500–5,000 units/kg in single doses of 50–100 units, intravenously or subcutaneously, protected 50–100% against *S. schottmuelleri*, *S. aertrycke*, *E. coli*, *Sh. dysenteriae*;

no protection by 3,000 units against *Ser. marcescens, Pr. vulgaris, Staph. aureus, D. pneumoniae.*[15] Streptothricin, 1,900 units, applied directly to site of experimental infection, protected 60% against *Cl. perfringens;* prolonged survival time against *Clos. histolyticum.*[46]

Active against *Br. abortus* in chick embryos and guinea pigs[27]; against blastomycotic infections in chick embryos[41]; no activity against virus of epidemic influenza, feline pneumonitis, lymphogranuloma venereum; no activity against *Trep. equiperdum,* toxin of *Cl. welchii* or *Cl. tetani*[13, 15, 39]; no favorable effect on guinea pigs experimentally infected with *Myco. tuberculosis hominis* by administration of a total of 13,125 units subcutaneously[48]; marked reduction of coliform bacteria in cecal contents of intestinal tract of chicks[42]; effective against exoerythrocytic forms of *Plas. gallinaceum* in chick tissue culture at 10 units/ml.[43]

Activity in vivo not markedly affected by number of organisms in experimental infecting dose; with time interval of 1–10 hours between experimental infection and therapy, protection still possible by streptothricin.[13]

PHARMACOLOGY

Toxicity. In mice, acute MLD units/kg, using streptothricin of 5–300 *E. coli* units per mg[13, 15, 21]:

intravenous:	500,000–750,000
subcutaneous:	250,000–750,000
oral:	750,000

In mice, using streptothricin preparations varying in potency from 84.5–529 units per mg, with dosages from 80–4,140 units administered intravenously, the immediate mortality ranged from 20.8–100% and delayed mortality among the survivors from 50.8–88.9%.[17]

In mice, the acute toxicity on intravenous administration decreased with increased purity of streptothricin:

Units/mg	Acute LD 50 (units/20 g)
84.5	286
250	1,728
330	2,400
529	2,630

The delayed LD 50 remained at about the same level for the purest and least pure material.[17]

In mice, intravenous administration of streptothricin caused lesions and sometimes gangrene of the small intestine, acute arteritis, myocarditis, lesions in kidney tubules, liver and nervous system; subcutaneously, caused local reactions in abdominal wall and kidney lesions; orally, by stomach tube, and by intraperitoneal administration, caused toxic symptoms, anorexia and convulsions.[44]

In rabbits, streptothricin sulfate or hydrochloride, intravenously, 5,000–20,000 units/kg twice a day caused destruction of mucous lining of stomach, mottled liver and kidney, and death; orally, 120,000–170,000 units per day, or topically on scarified skin, 30,000 units per day caused similar pathology; no toxicity on topical application to unbroken skin; intradermally, 2,000–6,000 units caused hemorrhagic areas.[47]

In cat and monkey, 50,000–100,000 units applied to the cerebral cortex caused twitching and convulsions.[45]

In chick, 10,000 units added to the basic diet was toxic and was not counteracted by liver fractions and folic acid [42]; in chick embryo, streptothricin hydrochloride was irritating.[41]

Excretion. Large proportion of administered streptothricin excreted by kidney.[13]

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SUBTENOLIN

First described by Hirschhorn, Bucca and Thayer in 1948[1]; derived from *Bacillus subtilis;* name suggested by enolic properties of this antibiotic.

PRODUCTION

Fermentation. Surface growth on medium containing *dl*-alanine 0.3%, potassium dihydrogen phosphate 0.5%, magnesium citrate·$14H_2O$ 0.25%, magnesium sulfate·$7H_2O$ 0.05%, glycerol 2.0%, manganous sulfate·$5H_2O$

0.0004%, cupric sulfate$\cdot 5H_2O$ 0.00005%; inoculated with spore suspension, incubated 3–4 days at 36° C, final pH 6.7–6.8.[1]

Isolation. Activated carbon added to culture filtrate, filtered, carbon cake washed with water, eluted with methanol; methanol eluate concentrated in vacuo; butanone added, precipitating the crude subtenolin, which is washed with butenone and ether, dissolved in methanol, and filtered; methanol solution concentrated to small volume in vacuo, inactive precipitate removed; methanol solution evaporated to dryness in vacuo; yield about 1.0 g from 10 liters of culture liquor, representing 25–50% recovery, final product having potency of 1,000–1,600 *Staph. aureus* dilution units per mg.[2]

CHEMISTRY

Properties. A light yellow powder.[2]

Constitution. C 51.4, H 7.00, N 7.88, S 0.81; soluble in water, ethylene glycol, 95% methanol, and many organic solvents containing a small amount of water; insoluble in acetone, butanone, ether and 95% ethanol. Somewhat hygroscopic; dialyzes readily through cellophane membranes, diffuses through agar. Ultraviolet spectrum shows peak at 2,700Å and a minimum at 2,500Å.[2]

Reactions. Not precipitated by acids or alkalies; thermostable in aqueous solution; stable at pH 2.0 for 18 hours at room temperature; loss of activity by drying in presence of water; positive test for peroxidase; positive enol, Molisch, ninhydrin, Benedict; negative Millon, Hopkins-Cole, trichloroacetic; dark brown with ferric chloride; reduces permanganate, iodine, bromine, ammoniacal silver nitrate, Shaffer-Somogyi sugar reagent; forms a crystalline hydrazone with 2,4-dinitrophenylhydrazine in alcoholic solution; with picric acid forms a brown solution and rosette crystals which are inactive.[2]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus.*[1]

Spectrum in vitro. Inhibition concentration (units/ml):[1]

Staph. aureus	140–200	*E. coli* (3 strains)	370–1,500
Staph. albus	140	*Sal. schottmuelleri*	600
Strep. sp.	375	*Past. pestis*	16.5
Strep. pyogenes	1,500	*Past.* sp.	22.5
Clos. novyi	300	*Clos. histolyticum*	600
Clos. perfringens	300	*Clos. oedematis-maligni*	770
Clos. fallax	500	*Eb. typhi*	165
Clos. tertium	500	*Neis. gonorrheae* (2 strains)	600
Myco. tuberculosis	>2,000		

No activity against: *Staph. aureus* (3 strains), *B. anthracis*, *B. subtilis*, *Micro. conglomeratus*, *Micro. lysodeikticus*, *S. enteriditis*, *S. paratyphi*, *S. typhimurium*, *Serratia marcescens*, *Aero. aerogenes*, *K. pneumoniae*, *D. pneumoniae*, *Eb. typhi*, *Ps. aeruginosa*, *Br. abortus*, *Br. melitensis*, *Br. suis*.[1]

Antibacterial action is reduced by complex organic substances, such as defibrinated rabbit blood, peptones. In nutrient broth, activity is reduced at 22 hours, but not at 5 hours.[1]

PHARMACOLOGY

LD 50 for mice: 30–60 mg (1,000 units/mg) intraperitoneally.

Excretion of subtenolin began 15 minutes after injection and continued to be secreted for 10 hours, 30–50% of the administered dose being detected in the urine.[1]

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SUBTILIN

First observed by Humfeld and Feustel in 1943 [1]; described and named by Jansen and Hirschman in 1944,[2] derived from *Bacillus subtilis*. SUBTILIN C, described by Hassall in 1948 [3] and also derived from *B. subtilis*, shows no color reaction with ferric chloride, but in other respects is identical with subtilin.[3]

PRODUCTION

Fermentation. Surface growth on medium containing (A) g/l $MgSO_4$ 0.5, KCl 0.5, K_2HPO_4 1.0, sucrose 30.0, asparagine 0.3%, $CuSO_4$ 0.04 ppm, $FeSO_4$ 0.03 ppm, $MnSO_4$ 1.0 ppm; incubated at 35° C for 3–6 days, final pH 5.3–6.0; yield up to 20,000 *M. conglomeratus*, 2,000,000 *L. casei* or 640 *Staph. aureus* units/ml.[2] (B) g/l sucrose 100, asparagine 2, glutamic acid 2, citric acid 0.5, Na_2SO_4 4.0, $(NH_4)_2PO_4$ 8.0, NaCl 0.3, Zn 2.0 ppm, Fe 4.0 ppm, Mn 50 ppm, Mg 50 ppm, K 100 ppm; incubated for 60–72 hours; yield 600–800 mg/l.[4] (C) Beet molasses 20% (solids), $(NH_4)_2HPO_4$ 0.8%, Mn 50 ppm; incubated at 35–41° C for 60 hours; yield 1.2–2.0 g/l.[5] (D) Asparagus butt juice, corn-steep liquor or molasses wort.[5]

Submerged growth in laboratory fermenter on medium containing (A) g/l sucrose 100, citric acid 11.7, Na_2SO_4 4.0, yeast extract 5.0, $(NH_4)_2HPO_4$ 4.2, essential salts; incubated for 10 hrs; 1,550 RPM, ratio air volume per minute per volume of medium 1.0. "Vegefat Y" antifoam: yield 1,500–2,000 mg/l.[6] (B) g/l asparagus butt juice concentrate (68% solids) 147;

incubated 10 hrs; yield 1.53 g/l.[7] (C) g/l beet molasses (82% solids) 250, $(NH_4)_2PO_4$ 8.0, $MnSO_4 \cdot H_2O$ 0.15; incubated at 35° C for 14 hrs; yield 1.23 g/l.[7]

Submerged culture in stainless steel 75-gal. fermenter on medium (A) above; incubated at 35° C for 10–12 hrs at 360 RPM; yield 900–1,200 mg/l.[6, 8]

For subtilin production, zinc, potassium, magnesium, manganese, iron were essential, citrate beneficial, calcium deleterious.[4, 9, 10]

Fermentation of subtilin C. Submerged culture on medium containing corn-steep liquor and sucrose.[3]

Isolation. *Surface pellicle:* (A) Extracted with ethanol, 1.0% aqueous acetic acid added to alcohol extract and the mixture concentrated *in vacuo;* the precipitate centrifuged, washed and freeze-dried.[11] (B) Extracted with *n*-butanol at pH 2.0–2.5, the butanol extract concentrated *in vacuo,* the precipitate freeze-dried.[11] (C) Acidified culture liquor extracted with *n*-butanol, petroleum ether added to the butanol extract and the mixture extracted with 1.0% aqueous acetic acid; sodium chloride added to aqueous extract, the precipitate washed with petroleum ether, excess saline removed and freeze-dried.[12, 13] (D) Culture liquor adjusted to pH 2.0 and extracted with *n*-butanol, aqueous sodium hydroxide added to butanol extract to pH 5.0; excess solid sodium chloride added, precipitating crude subtilin; the precipitate washed with dry butanol and with 10% aqueous sodium chloride.[14, 15]

Isolation of subtilin C. Culture liquor centrifuged, the precipitate freeze-dried, extracted or autoclaved with 80% aqueous alcohol; the alcohol extract diluted with water at pH 2.3, concentrated and freeze-dried.[3]

Purification. (A) Crude subtilin washed in 95% ethanol, extracted with 85% ethanol containing 1.0% acetic acid and 1.0% sodium chloride, the extracts discarded; the residue then extracted with pH 4.5 acetate buffer, the buffer extract de-ionized with ion-exchange resin, concentrated and freeze-dried.[11] (B) Crude subtilin extracted with 95% ethanol and then with 85% ethanol containing 1.0% sodium chloride and 1.0% acetic acid, the extracts discarded; the residue dissolved in water, sodium hydroxide to pH 4.5 and 0.4% sodium chloride added, the precipitate dissolved in water, reprecipitated and dissolved 3 or 4 times; the final solution de-ionized with ion-exchange resin and freeze-dried, or precipitated from 10% sodium chloride, dissolved in water, de-ionized and freeze-dried; yield 40 60%.[12, 13, 15]

CHEMISTRY

Constitution. Basic polypeptide; N 15.8%, S 4.2%, P trace.[11]

Properties. Amorphous white powder; soluble in water, in water-saturated *n*-butanol, 0–80% ethanol, and in methanol; solubility in water decreased when sodium chloride is present; insoluble anhydrous ethanol, butanol, amyl alcohol, acetone, ether, petroleum ether, chloroform; diffused through cellophane; levorotatory.[11]

Reactions. Stable in acid solution at pH 2.5; inactivated by light, alkali, pepsin, trypsin, methanol and formaldehyde [2, 11]; formed water-soluble salts with acids; blue color with ferric chloride; SUBTILIN C gave no color with ferric chloride.[2, 3]

Derivatives. Methanol and hydroxyethyl ester of subtilin showed increased activity against *Micro. conglomeratus* and *St. fecalis,* decreased activity toward *Staph. aureus;* ethyl and hydroxypropyl esters showed decreased activity [16]; methylated subtilin showed 2, 4 and 5 times activity against *Staph. aureus, Micro. conglomeratus* and *St. fecalis,* respectively [17]; methylated, ethylated and glycolated derivates showed 1–2 times activity against *Myco. tuberculosis.*[18]

BACTERIOLOGY

Assay. Serial dilution with *Staph. aureus, Micro. conglomeratus* and *Lactobacillus casei* [2, 3]; paper disc-plate with *B. cereus* [19]; turbidimetric with *Staph. aureus, Micro. conglomeratus* and *Lactobacillus casei* [5, 11, 20]; color reaction with ferric chloride.[2]

Spectrum in vitro. Inhibition of *Micro. conglomeratus* at 0.06–1.0 γ/ml,[2] *Trep. pallidum* at 2–3.5 γ/ml, *St. pyogenes* at 0.034 γ/ml [22]; inhibition dilution (× 1,000) against *Trypanosoma equiperdum* 2, *Endameba histolytica* 400, *Lactobacillus plantarum* 80, no activity against *Leishmania donovani, Ascaris suis* [21]; bacteriostatic to *Myco. tuberculosis* at 1:400,000, bactericidal at 1:20,000 [25, 26, 27, 28]; active against gram-positive bacteria, *N. gonorrheae, N. catarrhalis,* and some pathogenic higher fungi.[23, 24]

SUBTILIN C, 50% inhibition dilution (× 1,000)[3]:

Sar. lutea	10,000	*Micro. conglomeratus*	600
Staph. aureus	80–8,000	*Mycobacterium* sp.	300
C. xerosis	5,000	*Myco. phlei*	60
Lacto. helveticus	2,400		

Factors affecting activity. Most active at pH 2.2 [29]; better activity with urea and pectin in distilled water [30]; synergistic with BAL against pathogenic mycobacteria, slightly synergistic against nonpathogenic mycobacteria, no synergism with *Micro. lysodeikticus* [31]; activity against *Myco. tuberculosis* enhanced by sorbitan monooleate.[32, 33]

Resistance. Resistant strains of *Staph. aureus, Myco. phlei* and *E. communis* developed by serial transfer.[34]

Spectrum in vivo. Subtilin protected mice and guinea pigs against experimental infections with pneumococcus III, *B. anthracis, St. pyogenes* and *Staph. aureus.*[23, 29] Guinea pigs were protected against *B. anthracis* infection by 6–10 mg intraperitoneally, administered 9 hrs after infection [36]; mice were protected by 0.05 mg every 4 hrs against pneumococcus III,[35] and by 0.4 mg against *St. pyogenes*.[37] In guinea pigs, experimentally infected with *Myco. tuberculosis*, 22 mg/day subcutaneously had a pronounced therapeutic effect.[29, 30] In guinea pigs, mice, rabbits and hamsters, with experimental tuberculosis, no difference was observed between the subtilin-treated and the control animals.[27, 32, 33] Subtilin was ineffective against *Trep. pallidum* in rabbits,[22] *Trep. equiperdum* in mice,[21] and against influenza virus.[38]

Subtilin gave some protection for barley seed experimentally infected with *Xanthomonas translucens*.[39]

PHARMACOLOGY

Toxicity. In mice, LD 50 mg/kg: subcutaneously 2,500–3,000,[13] 670 ± 30,[21] intravenously 60 ± 3 [21]; intragastrically, 5 g/kg was lethal.[21] In embryonic chick heart tissue, subtilin at 1:500 was toxic.[40]

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SUBTILIN C See SUBTILIN

SUBTILYSIN

First described by Vallee in 1945; derived from *Bacillus subtilis*.

Produced on glucose nutrient broth, incubated for 20 days, final pH 9–10; heat-labile; active against *E. coli*, *V. comma*, *Cl. edematiens*, *S. gartneri* and *Pasteurella;* action similar to ACTINOMYCETIN.

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SULFACTIN

First described by Junowicz-Kocholaty, Kocholaty and Kelner in 1947; derived from *Actinomyces* sp., named from high sulfur content obtained by analysis of the crystalline sulfactin.[1]

PRODUCTION

Fermentation. Submerged culture in shaker flasks on medium containing g/l soluble starch 10, "Tryptone" 5, K_2HPO_4 2, NaCl 2, $FeSO_4 \cdot 7H_2O$ 0.01; incubated 4–7 days at 28–30° C, pH increased from 7.0 to 8.2; yield 1,000–4,000 *Staph. aureus* dilution units/ml.[1]

Isolation. Neutralized culture filtrate extracted with *n*-butanol, evaporated to dryness in vacuo, the residue extracted with boiling ether to remove a gummy yellow impurity, then extracted into chloroform. Anomalously, the chloroform extract contains about 70% of the original activity, whereas the *n*-butanol extract contains 50%.[1]

Purification. Chloroform extract chromatographed on a "Florisil" column previously washed with chloroform, eluted with 10% ethanol with 93% recovery, eluates evaporated to dryness in vacuo, residue extracted with chloroform, crystallized by concentration or by addition of ethanol; recrystallized.[1]

CHEMISTRY

Constitution. Proposed $C_{38}H_{55}N_{11}O_7S_4$ or $C_{27}H_{40}O_5N_8S_3$; found C 50.15, 50.40, H 6.17, 5.92, N 17.20, 17.22, S 14.01, 14.22.[1]

Properties. White double-pyramid crystals from chloroform, needles from alcohol; m.p. 245–275° C (cor. decomp.); soluble in chloroform, ethanol, ethyl acetate, dioxan, *n*-butanol; slightly soluble in methanol; almost insoluble in water, ether, petroleum ether, benzene; stable in boiling alcohol, 50% loss by boiling neutralized culture filtrate; diffuses through cellophane with loss of activity.[1]

Reactions. Reduces Fehling and permanganate; negative Sakaguchi, Molisch, biuret, ferric chloride.[1]

BACTERIOLOGY

Assay. Agar streak dilution method with *Staph. aureus:* 0.0254–0.027 γ of crystalline sulfactin equals one *Staph. aureus* unit; serial dilution with *Staph. aureus:* 0.0048 γ equals one unit.[1, 2]

Spectrum in vitro. Inhibition concentration (units/ml) [2]:

Coryne. diphtheriae	0.03	*Aero. aerogenes*	>32
Coryne. xerose	0.06	*Al. fecalis*	>32
Micro. roseus	0.125	*Br. abortus*	>32
Micro. lysodeikticus	0.25	*Br. melitensis*	>32
Micro. aurantiacus	0.25	*Br. suis*	>32
Neis. sicca	0.5	*Chromo. violaceum*	>32
Gaff. tetragena	0.5, 8	*Eb. typhi*	>32
Dip. pneumoniae III	0.5	*E. coli*	>32
B. subtilis	0.5, 1, 4	*E. communior*	>32
B. mycoides	0.5, 2, 16	*E. neapolitana*	>32
B. mesentericus	0.5	*K. pneumoniae*	>32
Staph. aureus	1	*Myco. smegmatis*	>32
Dip. pneumoniae I	1	*Neis. catarrhalis*	>32
B. circulans	2	*Pr. vulgaris*	>32
B. megatherium	2	*Ps. aeruginosa*	>32
Strep. pyogenes	4	*Sal enteritidis*	>32
B. anthracis	8, 16	*Sal. paratyphi*	>32
B. cereus	16	*Sal. schottmuelleri*	>32
Alpha Streptococcus	32	*Sarcina lutea*	>32
Gamma streptococcus	32	*Shig. dysenteriae*	>32

Shig. paradysenteriae	>32
Strep. fecalis	>32
Ser. marcescens	>32
Trich. interdigitale	>32
Vibrio comma	>32

Factors affecting activity. Activity is lowered 90% by 10% defibrinated horse blood.[2]

Spectrum in vivo. Mice experimentally infected with a fatal dose of *D. pneumoniae* type I, were 50–100% protected by intraperitoneal administration of 1–10 γ of sulfactin.[2]

PHARMACOLOGY

The LD 50 for mice, administered intraperitoneally, was approximately 2.75 mg.[2]

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TARDIN

First observed by Wilkins and Harris in 1943 [1]; isolated and named by Borodin, Philpot and Florey [2]; derived from *Penicillium tardum*.

PRODUCTION

Fermentation. Surface culture on 2% malt extract in tap water at 24° C, maximum yield at 9 days.

Isolation. Filtered culture medium adjusted to pH 7, extracted with amyl acetate, amyl acetate extract distilled in vacuo; brown sticky residue dissolved in benzene, insolubles removed by centrifuging.

Purification. Benzene solution passed through aluminum oxide column treated with HCl to adjust to pH 5.[2]

CHEMISTRY

Constitution. Proposed $C_{11}H_{16}O_3$: C 67.8, H 7.7, O 24.6; found C 68.2, H 7.58, O 24.22.

Properties. A pale yellow oil; slightly soluble in water, warm hexane; freely soluble in alcohol, acetone, ether, benzene, amyl acetate; $[\alpha]_{20}^{D} = -11.4$.

Reactions. Stable from pH 2 to 8, unstable above pH 8; with $FeCl_3$ gives purple color becoming brown on warming; no reaction with 2,4-dinitrophenylhydrazine. On decomposition or acid hydrolysis yields an acid and a neutral fraction.[2]

BACTERIOLOGY

Assay. Cylinder-plate method using *Staph. aureus*.[2]

Spectrum in vitro. Inhibition dilution (× 1,000):[2]

Staph. aureus	128
Strep. viridans	8
C. diphtheriae gravis	8
C. xerosis	64
Sal. enteritidis	1
E. coli	<1
Ps. aeruginosa	<1
Sabouraudites audouini	5
Sabouraudites lanosus	5
Trichophyton sabouraudii	5
Trichophyton equinus	5
Trichophyton lacticolor	<5
Trichophyton tonsurans	<5
Endomycopsis albicans	<5

Bacterial action is bactericidal; activity reduced by serum.[2]

PHARMACOLOGY

Toxicity. In mice 0.8 mg intravenously and 1.8 mg subcutaneously was tolerated, but edema and sloughing took place several hours later at the site of injection; human leucocytes in 1:4,000 tardin showed 100% mortality in one hour.

Physiology. Isolated guinea-pig uterus in 1:25,000 tardin showed increased contraction rate, then irreversible cessation; perfused heart stopped irreversibly at 1:25,000; slight to no effect on blood pressure of cat by 26–30 mg.[2]

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TERRAMYCIN

First described by Finlay *et al.* in 1950[1]; derived from *Streptomyces rimosus.*

PRODUCTION

Isolation. Culture filtrate extracted with *n*-butanol, butanol phase extracted with dilute acid to yield a crude aqueous concentrate.[2]

Purification. (A) Chromatography on alumina followed by repetition of solvent extraction and re-extraction with dilute acid; aqueous extract concentrated, yielding crude crystalline terramycin; crystals dissolved in dilute acid, then neutralized, to yield pure crystalline terramycin dihydrate.

(B) Hydrohalide salt prepared by hydrochloric acid in methanol, crystals dissolved in methanol and precipitated by addition of a miscible solvent.[2]

CHEMISTRY

Constitution. By analysis, C 53.05, H 5.91, N 5.64, O 35.4 (by difference) [1]; proposed $C_{22}H_{24-26}N_2O_9 \cdot 2H_2O$.[2] M.W. 440 (ebullioscopic), equivalent weight 460 (by titration.[2])

Properties. Several crystalline forms, e.g., needles, thick hexagonal plates, $\alpha = 1.636 \pm 0.004$, $\beta = 1.64444 \pm 0.004$, $\gamma = 1.700$ [1, 2]; m.p. 185° C (dec.). Isoelectric terramycin slightly soluble in water (0.25–0.3 mg. per ml at 25° C); neutral terramycin, the hydrochloride and the sodium salt soluble in acetone, methanol, propylene glycol, ethanol and other polar organic solvents; the salts quite soluble in water; insoluble in ether, petroleum ether. Optical rotation $[\alpha]_D^{25} = -196.6°$ (c = 1% 0.1N HCl), −2.1° (c = 1% 0.1N NaOH), +26.5° (c = 1% methanol). Ultraviolet maxima at ca. 247, 275 and 353 mμ (0.1 molar phosphate buffer pH 4.5). More than twenty characteristic absorption bands at 650–2600 cm^{-1} by infrared spectrum.[1, 2]

Reactions. Amphoteric, forming crystalline acid and basic salts; yellow disodium salt decomposes in solution at room temperature; acid salts in solution above pH 1.0 slowly hydrolyze depositing terramycin crystals; dry crystals stable for four days at 100° C, solutions at pH 1.0–2.5 stable for 30 days at 25° C; at pH 7.0, half-life at approximately 7 days; inactivation at higher pH.[2]

Positive ferric chloride, Pauly, Friedel-Crafts, Fehling and Molisch.[2]

BACTERIOLOGY

Assay. Cylinder plate with *Bacillus cereus* [3]; serial dilution.[4]

Spectrum in vitro. Inhibition concentration (γ/ml) [1]:

A. aerogenes	1.0	*Shig. paradysenteriae*	1.0
K. pneumoniae	3.0	*B. subtilis*	3.0
E. coli	5.0	*Staph. albus*	1.0
S. typhi	3.0	*Staph. aureus*	1.0
S. schottmuelleri	1.0	*Br. bronchiseptica*	3.0
S. paratyphi	1.0	*Ps. aeruginosa*	100
S. pullorum	10.0	*Proteus* sp.	1000

Twenty-five strains of pneumococci inhibited at 0.1–0.2 γ/ml [5]; active against several *H. pertussis* strains,[6] against *Endameba histolytica* and associated bacterial flora.[7] No activity against type A or B influenza virus by neutral terramycin [8]; no activity against *Pr. vulgaris*.[4, 10]

Mode of action. Primarily bacteriostatic, bactericidal at high concentrations relative to bacterial population.[9, 11]

Factors affecting activity. Affected by binding and inactivation by serum, by slow deterioration in culture medium[4]; not affected by bacterial population from 10^{-2} to 10^{-5} or by pH from 5.5–8.2[4, 11]; activity inversely proportional to number of organisms.[10]

Resistance. Strains of hemolytic coagulase positive *Staph. aureus* were generally resistant; serial transfer in terramycin-containing media induced resistance in enterococcus, *Staph. aureus,* coliforms and enteric organisms, but not in pneumococci or group A streptococci.[11]

Spectrum in vivo. *Experimental infections in animals.* In mice, terramycin was effective against pneumococcus I, hemolytic streptococcus A, *K. pneumoniae* A and *H. influenzae;* was prophylactic and therapeutic against spores of *Cl. septicum* or *Cl. tetani*[4]; effective against brucellosis, particularly in synergism with streptomycin,[12] and against *Past. tularensis.*[21] Terramycin caused reduced mortality from septicemia with flora of the intestinal tract, following x-ray irradiation at over LD 50 concentration.[13]

In mice, cotton rats and chick embryos, terramycin was effective against *Rickettsia prowazeki, R. mooseri, R. tsutsugamushi,* rickettsialpox, *Dermacentrogenus rickettsi,* scrub typhus and Rocky Mountain spotted fever; in mice and cotton rats, definite activity against murine and epidemic typhus respectively[14]; in mice and chick embryos, effective against *R. akari.*[15]

In chick embryos, terramycin protected against *R. tsutsugamushi, R. burneti, R. rickettsii,*[15] against *Hemophilus pertussis*[6] and against psittacosis virus in preliminary trials.[8]

In dogs, favorable results were obtained with terramycin therapy of experimental peritonitis.[17]

In guinea pigs, preliminary results on tuberculosis therapy with terramycin were favorable.[18]

Terramycin was ineffective against influenza virus, rabies virus and herpes simplex in mice and chick embryo.[8, 19]

Clinical. Good responses were obtained in urinary tract infections with *E. coli, Aero. aerogenes, Strep. fecalis,* nonhemolytic streptococcus and other susceptible organisms,[3, 20] with 2-6 g/day orally; sterilization of urine was produced in a few instances, but generally persistence of coliforms or replacement by *Proteus* or *Staphylococcus* occurred.[10, 11] Favorable results occurred with pneumonias caused by *K. pneumoniae, D. pneumoniae* and in mixed bacterial pneumonias[5, 20, 22, 23] with oral dose 1.25 g/6 hrs. to 1.0 g three times daily[23]; favorable effect in primary atypical pneumonias[20, 24]; good response in pneumonia, but somewhat delayed and flora of sputum replaced by *Staphylococcus,* by 2–6 g/day orally.[11]

Terramycin therapy was effective in brucellosis,[12] scrub typhus,[16] rickettsialpox,[15] pneumococcal meningitis,[21] influenza virus,[8] *H. pertussis,*[6] acute follicular tonsillitis, septic sore throat, septicemia by *E. coli* and *Bacteroides,* and subacute bacterial endocarditis.[20]

Fecal flora susceptible to terramycin: *Clostridium, Staphylococcus, Streptococcus, Echerichia, Klebsiella, Aerobacter, Salmonella* and *Shigella.*[10] At 1–2 g/day for 10 days, terramycin caused disappearance of *Endameba histolytica* in a majority of patients, and elimination of other intestinal amoebae.[7]

Terramycin was ineffective against *Pseudomonas, Proteus, Staphylococcus, Salmonella* infections [3, 21]; in fecal flora, *Pseudomonas* was moderately resistant, *Proteus* was resistant and tended to increase in number as other genera decreased.[10, 11, 13] Failure of terramycin was reported in typhoid and salmonellosis,[21] acute endocarditis caused by *Micro. pyogenes* and *E. coli,*[20] against intestinal helminths and a flagellate,[7] and against tuberculous pneumonia.[3]

PHARMACOLOGY

Toxicity. Terramycin·HClLD50(mg/kg):

mice	intravenous	150–200,[3] 178 [25]
	subcutaneous	650,[3] 892 [25]
	oral	7200 [25]
rats	intravenous	260 [25]

Terramycin·HCl, in mice 100–200 mg/kg/day subcutaneously was tolerated for 2 weeks [3]; in cats, 186 mg/day orally was tolerated for 10 weeks; in dogs, 465 mg/day orally was tolerated for 8 weeks, and 47–93 mg/kg/day intramuscularly, for 9 weeks.[25]

Sodium terramycin, in mice orally had no effect on the growth curve,[25] caused renal impairment [3]; in dogs, 370 mg/day orally was tolerated for 8 weeks and 38–75 mg/kg/day intramuscularly for 9 weeks, with no effect on liver and renal function, urinanalysis, blood analysis, blood pressure, respiration and contraction of isolated smooth muscle; in dogs, 75–225 mg/kg/day intramuscularly caused eventual death, with liver and renal function impairment; in cats, 38 mg/day intramuscularly for 9–10 weeks was tolerated.[25]

In dogs, terramycin caused occasional vomiting and diarrhea.[25]

In clinical trials, 2–4 g/day orally of terramycin hydrochloride showed no evidence of hematologic, renal, hepatic or central nervous system damage; nausea, vomiting and diarrhea occurred in approximately 40 per cent of the patients; in one patient, a dermatologic reaction was observed.[3]

Blood levels. Clinically, 2.0 g of terramycin·HCl orally gave a detectable blood level (0.007 γ/ml) after one-half hour, 4.0–5.0 γ/ml at 2–6 hours, and persisted in the blood stream for 24 hours [3]; 1.0–1.25 g every 6 hours maintained a therapeutic level in the blood[19]; 0.25 g every 6 hours maintained significant blood levels.[26]

Absorption, Diffusion, Excretion. Terramycin was readily absorbed after oral administration to give significant blood levels for one to 24 hours[19, 25]; not absorbed rectally.[19] Diffusion occurred rapidly into the tissues and organs of the body, into the pleura and across the placenta but not into the intact central nervous system.[3, 26] Urine levels of 50–300 γ/ml were detected[3] and recovery ranged from 11–48 per cent.[3, 25, 26]

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TOMATIN See TOMATINE

TOMATINE

First described by Irving, Fontaine and Doolittle in 1945[1]; named LYCOPERSICIN, renamed TOMATIN to describe the relatively impure antibiotic[2]; TOMATINE to describe the crystalline antibiotic[3]; derived from *Lycopersicon pimpinellifolium* (Red Currant tomato plant).

PRODUCTION

Isolation. (A) Dried plant extracted with hot methanol, extract evaporated in vacuo to dryness; residue extracted with boiling water; yield 21 g or 4,000 arbitrary units from 200 g dried plant.[4] (B) Plant autoclaved, extract centrifuged, evaporated in vacuo to dryness; residue extracted in absolute methanol, extract evaporated in vacuo to dryness; residue dissolved in water.[2] (C) Leaves extracted with 95% ethanol, the extract concentrated to a syrup; the residue extracted with boiling water and the aqueous extract concentrated.[3]

Purification. Crude concentrate adjusted to pH 10.0 with ammonia, precipitate removed; the precipitate suspended in water, dissolved by addition of HCl to pH 4.0; reprecipitated with ammonia, precipitate dissolved in HCl solution; reprecipitated and precipitate dissolved in hot ethanol; water added to dilute alcohol solution to 70% and the solution chilled at 4° C for 2 days; the amorphous precipitate removed, dissolved in hot ethanol and evaporated in vacuo to dryness; residue dissolved in hot 1,4-dioxan (peroxide-free) and evaporated to dryness; this residue dissolved in warm 80% dioxan, made slightly alkaline with ammonia, centrifuged; the supernatant allowed to crystallize overnight; recrystallized from 80% ammoniacal dioxan, washed with 70% ethanol, with absolute ethanol and finally with ether; also recrystallized from 70% ethanol.[3]

CHEMISTRY

Constitution. Glycosidal alkaloid; by analysis, C 57.2, 57.9, H 8.15, 8.46, N 1.30, 1.42; M.W. minimum 1,050.[3]

Properties. Needles; m.p. 263–267° C (dec.); soluble in absolute methanol, ethanol, isopropanol, butanol, dioxan, propylene glycol; almost insoluble in water but soluble as hydrochloride; insoluble in ether, petroleum ether, aromatic and aliphatic hydrocarbons, ketones, esters, chloroform; adsorbed in slightly acid solution on activated carbon, eluted with acid aqueous methanol; levorotatory.[3, 4]

Reactions. Stable in strong alkali; readily hydrolyzed by boiling dilute acid to yield tomatidine hydrochloride and a carbohydrate; thermostable; negative phenol, ninhydrin and Lieberman for cholesterol; positive Molisch, orcinol; faint test for solanine.[3, 4]

BACTERIOLOGY

Assay. Cylinder plate with *Fusarium oxysporum f. lycopersici*[1, 3]; arbitrary unit producing 18.5 mm inhibition zone on standard assay plate.[4]

Spectrum in vitro. Inhibition concentration (mg/ml)[3]:

Achorion gypseum	0.25	*Trichophyton mentagrophytes*	0.5
Achorion schoenleinii	0.1	*Candida albicans*	0.1
Blastomyces dermatitidis	0.1	*Penicillium notatum*	>1.0
Epidermophyton floccosum	0.25	*E. coli*	>1.0
Microsporum audouini	0.1	*Staph. aureus*	>1.0
Fusarium oxysporum f. lycopersici	>1.0		

Inhibited by 1.0 unit/ml[4]:

Candida albicans
Cryptococcus neoformans
Debaryomyces histolytica
Trichophyton mentagrophytes
Trichophyton interdigitale
Trichophyton rubrum
Trichophyton gypseum
Epidermophyton floccosum
Microsporum audouini
Achorion gypseum
Achorion schoenleinii
Blastomyces dermatitidis
Coccidioides immitis
Histoplasma capsulatum

Tomatine inhibited *B. subtilis*, *Phytomonas solanacearum*, *Aspergillus clavatus*, *Fusarium oxysporum f. pisi* and *conglutinans*[2]; several bacterial species were only partially inhibited at 1.0 unit/ml.[4]

Factors affecting activity. Rutin and quercetin antagonized the activity of tomatine against *Candida albicans*[5]; rutin did not antagonize tomatine activity against *Fusarium oxysporum f. lycopersici.*[6]

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TRICHOTHECIN

First observed by Brian and Hemming in 1947 [1]; described by Freeman and Morrison in 1948 [2]; derived from *Trichothecium roseum.*

PRODUCTION

Fermentation. Surface growth on medium containing g/l ammonium tartrate 2, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 1.0, KCl 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, glucose (crude) 50, corn-steep liquor 10 ml, pH 5.0; incubated at 25° C for 12–28 days; yield up to 40–50 mg/l.[2, 3, 4]

Isolation. Culture filtrate extracted with chloroform, the chloroform extract evaporated *in vacuo* to dryness; the residue taken up in chloroform, excess ether added and the precipitate discarded; petroleum ether added to the filtrate, the precipitate discarded and the final filtrate evaporated to a yellow syrup.[4]

Purification. Chromatographed in ether over aluminum oxide, dissolved in petroleum ether-chloroform, cooled, a crystalline precipitate discarded and the filtrate evaporated to dryness; the residue dissolved in carbon tetrachloride, chromatographed and recrystallized from petroleum ether; yield 36 mg/l.[2, 4]

CHEMISTRY

Constitution. Proposed $C_{15}H_{20}O_4$, C 68.2, H 7.5, CCH_3 17.0, OCH_3 0.24, M.W. 264; or $C_{16}H_{18}O_4$, C 68.7, H 6.9, M.W. 262; found C 68.5, 68.7, 68.8, H 7.2, 7.3, 7.4, CCH_3 16.4, OCH_3 0.24, M.W. 278 (Rast), one ketone and one ethylene group, no free carboxyl, hydroxyl, alkoxyl or aldehyde.[2, 4]

Properties. Slender, colorless needles; m.p. 110° C [2]; m.p. 118° C (corr.) [4]: soluble in water (400 mg/l), chloroform, acetone, benzene, ethanol; slightly soluble in petroleum ether; $[\alpha]_D^{18} = +44°$ ($c = 1.0$ in chloroform); ultraviolet molar extinction 44 at 331 mμ, 10^4 at 220 mμ in chloroform or hexane; characteristic infrared spectrum.[2, 4]

Reactions. Neutral; *thermostable at pH 7; alkali-labile; reduced cold* permanganate but not bromine; hydrogenated over *palladium black* and platinum oxide; formed 2,4-dinitrophenylhydrazone.[2, 4]

BACTERIOLOGY

Assay. Cylinder plate; inhibition of germination of conidia of *P. digitatum*, complete inhibition at 1.25 γ/ml; of *Bot. allii* spores at 3.12–6.25 α/ml.[2, 3, 4]

Spectrum in vitro. Growth inhibition concentration (γ/ml)[3]:

A. fumigatus	80	*Penicillium notatum*	>80
A. niger	16	*Penicillium oxalicum*	16
Cephalosporium longisporum	80	*Penicillium roqueforti*	80
Cladosporium herbarum	16	*Penicillium spinulosum*	16
Fusarium graminearum	16	*Stachybortris atra*	>80
Helminthosporium sacchari	80	*Trichoderma viride*	>80
Paecilomyces varioti	80	*Trichothecium roseum*	>80
Penicillium ceseicola	80	*Chaetomium convolutum*	16
Penicillium citrinum	80	*Neurospora crassa*	3.2
Penicillium digitatum	0.04	*Sacch. carlsbergensis*	16
Penicillium expansum	80	*Mucor erectus*	80
Penicillium lilacinum	80	*Syncephalastrum racemosum*	80
Penicillium meleagrinus	16	*Thamnidium elegans*	16
P. nigricans-janczewskii	>80		

At 400 γ/ml, trichothecin was inactive against *Staph. aureus, B. subtilis, E. coli.*[2, 4]

Factors affecting activity. Not inactivated by cysteine.[4]

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TYROCIDINE See TYROTHRICIN

TYROTHRICIN

First described by Dubos in 1939 [1, 2]; derived from *Bacillus brevis*. Tyrothricin is a dual antibiotic, composed of the chemically and bacteriologically dissimilar antibiotics, GRAMICIDIN and TYROCIDINE. Tyrocidine hydrochloride was originally referred to as GRAMINIC ACID; and a crystalline mixture of tyrocidine and its hydrochloride as GRAMIDINIC ACID.[3, 4, 5]

An antibiotic closely resembling tyrothricin and derived from an organism either identical with or related to *B. brevis*, was described by Hoogerheide in 1940.[6, 7]

GRAMICIDIN S, an antibiotic related to tyrothricin and derived from an organism related to *B. brevis*, was described by Gause, Brazhnikova and Lissovskaya in 1944.[8, 9, 10]

PRODUCTION

Fermentation. Production is by surface growth on medium containing: (A) Acid-hydrolyzed casein 1.0% at pH 7.0; incubated at 37° C for 3–4 days, final pH 9.0; yield 0.2 g/l.[1, 11] (B) "Tryptone" 1.0%, NaCl 0.5%, at pH 7.0 or gelatin (impure) 1.0%, $MgSO_4$ 0.05%, KH_2PO_4 0.2%, Na_2HPO_4 0.4%, NaCl 0.5% at pH 7.0; incubated at 37° C for 6 days; yield up to 0.5 g/l.[12] (C) Nutrient broth diluted 1:2, peptone 10 g/l, leucine, serine and alanine 33 mg/l.[13] (D) Peptone 1%, NaCl 0.5%, yeast extract 0.3%, pH 6.5; incubated at 37° C for 3 days; yield 0.8 g/l.[17] (E) Asparagus-butt juice; corn-steep liquor; hydrolyzed soybean meal.[28]

Production is by submerged culture in 2 gal. glass-lined or iron aerated tanks on medium containing: (A) g/l glucose 10 (sterilized separately), K_2HPO_4 0.5, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 0.01, $MnSO_4 \cdot 4H_2O$ 0.01, $FeSO_4 \cdot 7H_2O$ 0.01, $Ca(H_2PO_4)_2 \cdot H_2O$ 2 ml of saturated solution, *d*-glutamic acid 5, pH 6.5; (*d*-glutamic acid may be replaced by *dl*-phenylalanine, *dl*-aspartic acid, *d*-alanine, hydroxyproline, serine, threonine, valine, leucine, proline, histidine, isoleucine, B-alanine, glycine); incubated for 35 hours, final pH 8.4; aeration 2 liters per minute for 5 liters of medium, 60 RPM, air pressure 0–15 psi; yield 162–180 mg/l.[14, 15, 16] (B) On a medium similar to the previous, with sodium glutamate as nitrogen source and with urea 0.1–2.0% or with thiourea 0.01–0.5%; yield with urea 150 mg/l, with thiourea 412 mg/l.[19] (C) In 10-l aspirators, g/l glucose 10, K_2HPO_4 0.5, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 0.01, $FeSO_4 \cdot 7H_2O$ 0.01, $CaCl_2$ 0.25, ammonium succinate 5, pH 6.8; incubated at 37° C for 150 hours, final pH 8.5; air ratio 1.0 liter air/min per liter culture medium; antifoam 2% octadecanol in lard oil; yield 0.3–0.53 g/l; with added biotin 0.005 mg/l, 0.88 g/l; in 200-l glass-lined tank, 0.3–0.4 g/l in 120 hours.[18] (D) In shaker flasks on tryptone medium.[28] (Tyrothricin from submerged culture gave 10% higher assay.[17])

Of GRAMICIDIN S, surface culture on medium containing yeast autolyzate 10%, glucose 0.5% incubated at 40–41° C for 6 days; yield 0.4–0.5 g/l: if peptone included in medium, yield 0.75 g/l.[8, 9, 10]

Isolation. *Of TYROTHRICIN:* (A) Culture filtrate acidified to pH 4.5 with HCl; precipitate dissolved in acid acetone, filtered and filtrate evaporated to dryness; residue washed with ether, taken up in alcohol and precipitated by addition of HCl; the precipitate removed, dried and taken up in dioxan; reprecipitated with HCl to yield a grayish powder.[1] (B) Culture liquor adjusted to pH 4.7 with HCl, filtered, precipitate dissolved in 95% alcohol; alcohol solution filtered, added to 10 volumes of 1% NaCl, precipitating tyrothricin.[11] The tyrothricin content of the culture liquor was found largely in the insoluble cells and debris, only a small proportion being contained in the clear supernatant.[20] (C) Culture liquor acidified to pH 4.1, centrifuged; precipitate extracted with ethanol, ethanol extract added to 10 volumes 1.0% NaCl; precipitate dried *in vacuo*, washed with anhydrous ether and redried *in vacuo*.[17]

Of GRAMICIDIN S: culture liquor acidified to pH 4.7 with HCl, filtered, the precipitate dried, dissolved in alcohol and "Seitz"-filtered.[8, 9]

Of GRAMICIDIN: (A) Tyrothricin extracted with acetone-ether 1:1, the extract evaporated to a brown gummy residue; the residue taken up in warm acetone, cooled and allowed to crystallize, yield 20% maximum, of which only one-half to two-thirds can be isolated as crystalline gramicidin.[5]

(B) Tyrothricin extracted with anhydrous acetone, the extract cooled and allowed to crystallize.[7] (C) Prolonged extraction of tyrothricin with ether.[21, 22] (D) Removal of tyrocidine by precipitation with phosphotungstic acid.[47]

Of TYROCIDINE: (A) Acetone-ether insoluble residue of tyrothricin dissolved in boiling absolute alcohol, the solution cooled, alcoholic HCl added and allowed to crystallize as the hydrochloride.[5, 23] (B) Precipitation with phosphotungstic acid.[47]

Purification. *Of GRAMICIDIN:* (A) Recrystallization from acetone or dioxan, washed with cold acetone-ether.[5] (B) Countercurrent distribution into two or more gramicidin-like components.[41]

Of TYROCIDINE: HCl, recrystallization from absolute methanol.[5]

Of GRAMICIDIN S: crude material washed thoroughly with ether, dissolved in warm alcohol, treated with activated carbon, cooled and allowed to crystallize; crystals washed with cold acetone.[8, 9]

CHEMISTRY

Constitution. *Of GRAMICIDIN:* a cyclopeptide:

Proposed	By Analysis
$C_{74}H_{106}N_{14}O_{14}$ [24]	C 62.7, H 7.5, N 13.9 [24]
$C_{55}H_{79}N_{11}O_{10}$ [25]	C 62.7, H 7.59, N 14.8 [5]
$C_{74}H_{105}N_{15}O_{18}$ [25]	C 62.7, H 7.4, N 14.8 [21]
$C_{146}H_{202}N_{30}O_{26}$ [26]	C 62.8, H 7.25, N 15.05 [26]

M.W. 1,200–1,600 (cryo. in camphor), 3,100 (isothermal distillation), 3,036 (flavianate), 908 (phenol), 589–1,220 (cyclohexanol) [21]; 1,400 (cryo. in camphor) [24]; 1,413 [25]; 2,790 (analysis), 3,100 (isothermal distillation in methanol), 2,400–4,500 (diffusion in butanol), 2,987–3,036 ± 300 (flavianate).[26]

Of TYROCIDINE·HCl: Proposed $C_{63}H_{83}N_{13}O_{13}\cdot HCl$ [25]; by analysis, C 59.6, H 6.66, N 14.31, C 12.7 [5]; C 59.4, H 6.8, N 13.5, O 2.7 [23]; M.W. 1,267.[25]

Of GRAMICIDIN S: M.W. 1,060–1,340.[29]

	Amino acid constituents		
	gramicidin	tyrocidine	gramicidin S
tryptophane	+	+	–
tryosine	–	+	–
dl-phenylalanine	–	+	±
proline	–	±	+
ornithine	–	+	+
dl-leucine	+	+	+
dl-valine	+	+	
hydroxyproline	–	–	
dl-alanine	+	+	
glycine	±	–	
glutamic acid	–	+	–
aspartic acid	–	+	– [25–27, 29–40]

About one-half of the α-amino acids of gramicidin are of the *d*-form, about one-fifth of the α-amino acids of tyrocidine are of the *d*-form, by *d*-amino oxidase method.[24, 27]

Properties. *Of GRAMICIDIN:* colorless platelets with pointed ends; m.p. 230–231° C,[5] 228–230° C [29]; soluble in lower alcohols, acetic acid, pyridine; moderately soluble in dry acetone, dioxan; almost insoluble in water, ether, hydrocarbons and chlorinated hydrocarbons [5]; $[\alpha]_D^{25} = +5°$ (c = 0.4% in 95% alcohol), +2.5° (c = 1.5% in absolute alcohol) [5]; ultraviolet maxima at 2,815, 2,910 and 2,690Å, $E^{1\%}_{1\,\mathrm{cm}}$ = 124 ± (at 2,815Å).[21]

Of TYROCIDINE·HCl: fine colorless needles or rods truncated at angle of 52° ± 5° with parallel extinction; m.p. 237–239° C,[23] ca. 240° C (dec.),[5] 240° C [29]; moderately soluble in methanol, ethanol, acetic acid and pyridine, solubility increased by addition of small amount of water; slightly soluble in water, acetone and dioxan; insoluble in ether and hydrocarbons[5]; $[\alpha]_D^{25} = -101°$ (c = 1% in 95% alcohol),[5] $-102°$.[23]

Of GRAMICIDIN S: thin colorless needles[8, 9]; n.p. 268–270° C[29]; soluble in ethanol, slightly soluble in acetone, insoluble in water, acids, alkali.[29]

Reactions. *Of GRAMICIDIN:* no amino reactions, no basic or acid groups in acetone or alcohol titrations; quantitatively acetylated with acetic anhydride in pyridine[25]; crystalline product with flavianic or rufianic acid in methanol; a gel with picric acid; no precipitation with chloroplatinic acid, phosphotungstic acid or gold chloride[21]; does not precipitate soluble proteins; thermostable; not digested by enzymes[5]; tyrothricin from submerged culture more stable than that from surface culture, forms more stable colloidal suspension in water.[17]

Of TYROCIDINE: positive ninhydrin but no carbon dioxide evolved; positive *p*-dimethylaminobenzaldehyde; red color with diazobenzenesulfonic acid; positive Millon; acetylated by acetic anhydride in pyridine[25]; precipitated with some soluble proteins, phosphotungstic acid; thermostable; not digested by enzymes.[5]

Of GRAMICIDIN S: positive ninhydrin, biuret; negative xanthoproteic, Millon, Pauly, Voisenet, Sakaguchi[29]; thermostable and acid-stable.[8, 9]

Derivatives. With formaldehyde, methylol derivative of gramicidin having reduced hemolytic properties, increased water-solubility, lower toxicity with little change in antibiotic activity; succinic acid derivatives[42–47]; with nitrite, chromic acid, bromine, iodine, hydroxylamine, HCl, NaOH and ammonium hydroxide, yielding derivatives with lower activity and reduced hemolytic properties.[48, 49]

BACTERIOLOGY

Assay. Serial dilution with *Streptococcus hemolyticus*,[50] with *St. fecalis*[55]; turbidimetric with *Micrococcus conglomeratus*[15, 21]; suppression of acid production by *St. lactis*[17]; hemolysis of rat red blood cells[51]; colorimetric by tryptophane estimation.[56]

Spectrum in vitro. Inhibition dilution (× 1,000):[52]

TYROTHRICIN

Staph. aureus	40	*S. paratyphi*	1.2
Staph. albus	40	*E. coli*	1.2
St. viridans	1,000	*Eb. typhi*	1.2
St. hemolyticus	1,000	*Achorion schoenleinii*	20
N. catarrhalis	1.6	*Microsporon gypseum*	10
Candida albicans	5	*Trichophyton gypseum*	10

Inhibition concentration (γ/ml)[53-59]:

	Gramicidin	Tyrocidine
pneumococcus	0.01–2.5	1.0
hemolyt. streptococcus	2.0–10	5.0
meningococcus	5.0	1.0
Past. avicida	500	——
Past. tularensis vir.	250	100
Past. tularensis avir.	25	25
Staph. aureus	10–>10	50
Shig. dysenteriae	>500	50
E. coli	500	500
S. schottmuelleri	500	250
Eb. typhi	500	500
N. gonorrheae	1.0	0.01
St. fecalis	>10	——
Leish. tropica	>1.0	5
Tryp. cruzi	>1.0	5
Tryp. lewisi	>1.0	5

Tyrothricin at 50 γ/ml was bactericidal to *Trichomonas vaginalis;* gramicidin and tyrocidine were ineffective against *Leptospira icterohemorrhagiae* and *Bartonella bacilloformis*[53]; tyrothricin at 6 mg % inhibited gram-positive oral bacteria, at 60 mg %, gram-negative forms were affected[60]; at 0.5 mg/ml inhibited luminescence of *Photo. fischeri*[62]; bacteriostatic and bacteriostatic to rhizobia.[69]

Inhibition concentration of Gramicidin S (γ/ml)[8, 9]:

Staph. aureus	10	*Eb. typhi*	50
Streptococcus	10	*S. paratyphi*	50
D. pneumoniae	10	*Shig. dysenteriae*	12
Cl. welchii	7	*V. comma*	25
Cl. histolyticum	7	*Pr. vulgaris*	100
E. coli	25		

Factors affecting activity. Effect of serum on tyrothricin activity varied with organism and culture medium used[3, 54, 61, 79, 88, 112]; tyrocidine antagonized by mucin[58]; gramicidin antagonized by lecithin, cephalin, spingomyelin, un-ionized and cationic detergents[63, 64]; synergism between tyrothricin and sulfadiazine[65]; synergism between gramicidin and penicillin[67, 68]; tyrothricin activity against gram-negative bacteria increased by protamine.[66]

Gramicidin S activity reduced by lipoids against gram-positive but not against gram-negative bacteria.[8, 9]

Mode of action. Bacteriostatic and rapidly bacteriocidal[50, 70, 71, 72]; increased oxygen uptake in *Staph. aureus* and increased lactic acid production by *St. hemolyticus* by gramicidin, depressing effect by tyrocidine[73]; antibiotic action increased progressively with increased exposure[77]; inhibition of metabolism and immobilization of bovine spermatozoa by gramicidin[74]; gramicidin and tyrocidine depress surface tension of water, increased by glycerin or glycerin plus alcohol.[75]

Resistance. Increased resistance to tyrothricin by some *Staph. aureus strains following therapy.*[76]

Spectrum in vivo. *Experimental infections in animals.* In mice, 1–2 mg tyrothricin intraperitoneally within a few hours of infection, protected against pneumococcus, but not against *K. pneumoniae; no protection against Cl. welchii* [78,80,81,88]*; no protection by intravenous, intramuscular* or subcutaneous administration [88]; tyrothricin orally had no effect on the fecal flora, but 80 mg/day with sulfasuxidine produced an almost sterile watery fecal mass.[85,86] In rabbits, 3–40 mg injected into the pleural cavity protected against *St. hemolyticus; Staph. aureus* and *Cl. welchii* more resistant [82,83,88]; no benefit in eye infection by tyrothricin suspended in saline.[84] In chickens, tyrothricin intravenously protected against *Plasmodium gallinaceum.*[87]

Veterinary. In the therapy of acute bovine mastitis, tyrothricin or gramicidin at dosages from 20–240 mg per quarter in 1–4 treatments, eradicated *St. agalactiae* and effected resumption of normal milk production in 80–93% of the cases treated; treatment less effective during lactation period.[89–101] Chronic mastitis and *St. uberis* infections were more resistant.[95,96] The vehicles preferred (from the standpoint of minimal irritation) were: aqueous-mineral oil emulsions,[93,95,97,99] sterile water suspensions,[91,94] aqueous-olive oil emulsion.[101]

Tissue culture. Tyrothricin at 60 γ/ml was bactericidal to *Staph. aureus, St. hemolyticus, St. fecalis* and *St. salivarius* without toxic effect.[107]

Clinical. Tyrothricin was effective by topical application in infections with *Staph. aureus, Staph. albus,* streptococcus and pneumococcus in eye infections, ulcers, skin infections; ineffective against *E. coli, Pr. vulgaris, Ps. aeruginosa, K. pneumoniae;* in sinus infections, tonsillitis, otitis, mastoiditis, empyema and cystitis conflicting results were obtained; resistant strains encountered after therapy [102–114]; scarlet fever carriers became non carriers following nose and throat spray with tyrothricin.[115]

Gramicidin S, topically, was used successfully in infections with staphylococcus, streptococcus, *Proteus* and *Ps. aeruginosa* [116]; good results in the therapy of peritonitis, impetigo, osteomyelitis, empyema, abscesses, burns and anaerobic infections.[117]

PHARMACOLOGY

Toxicity. In mice and rats, LD 50 mg/kg [88,118]:

intraperitoneal: 20–45 tyrothricin
40–60 gramicidin
40–60 tyrocidine

intravenous:	3.7 tyrothricin
	15–16 tyrocidine
	2.5–3.0 gramicidin
oral:	1,000 tyrothricin tolerated

In rabbits or dogs, tyrothricin or gramicidin 0.4–10 mg/kg/day intravenously, was lethal in a few days; 0.3 mg/kg/day caused ascites and fatty degeneration of the liver; in rabbits, 5 mg in the respiratory passage caused lung consolidation, 10 mg was sometimes fatal [118–120]; slight tissue reaction at site of injection, no effect when applied to conjunctival sac or rectally [88]; in guinea pigs, persistent nodules at site of injection [118]; administered into the pleural cavity, rabbits tolerated 80 mg and humans 200 mg of tyrothricin.[120]

Gramicidin was slightly hemolytic to rabbit and human red cells at 0.01–0.375 mg/ml; hemolysis antagonized by glucose in saline. Tyrocidine hemolyzed rabbit red cells at 0.07–0.005 γ/ml.[120, 123] Gramicidin was hemolytic to rabbit and sheep red cells at 0.005–0.01 mg/ml; hemolysis antagonized by serum and by glucose. Tyrocidine caused rapid hemolysis, antagonized by serum.[121, 122]

Tyrothricin cytolyzed leucocytes at 1:4,000; at 1:40 in the presence of serum [124]; inhibited tissue culture of rabbit lymph node 20–78% at 10–100 γ/ml; gramicidin inhibited 40–70% at 10–20 γ/ml; tyrocidine inhibited 5–32% at 100 γ/ml.[125, 126] Tyrothricin did not inhibit the growth of tissue culture.[127]

Tyrocidine lyzed protozoa, was lethal to fertilized frog eggs; gramicidin immobilized spermatozoa.[88]

Physiology. Tyrocidine caused increased contraction of isolated rabbit intestine and cessation of contractions in isolated frog heart; gramicidin was without effect.[88]

MISCELLANEOUS

Tyrothricin inhibited fibrinolysis caused by *B. hemolytic streptococcus* or its culture filtrate, and plasma coagulation by staphylococcus.[128] Deterioration of tetanus toxin is prevented by tyrothricin at 0.005–50 γ/ml; no effect upon the toxic action *in vivo*.[129]

Tyrothricin has been used as a component of selective culture media for the isolation of *Hemophilus influenzae* [130] and of *Neisseria gonorrheae*.[131]

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USNIC ACID

Antibiotic activity first described by Burkholder and Evans in 1945[1]; derived from many species of lichens including *Usnea barbata*,[2] *Cladonia sylvatica*,[3] *C. alpestris*,[4,5] *C. cristatella*,[6] *Ramalina reticulata*,[3] *Cetraria islandica*[7] and from species of the Evernia, Platysma, Parmelia, Lecanora, Haemotomma, Cladina, Alectoria, Squamaria and Diatora.[8] Usnic acid is produced by the fungal symbiont of the lichen.[6]

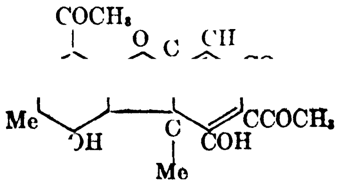
PRODUCTION

Fermentation with the fungal symbiont of *Cladonia cristatella* on synthetic medium.[6]

Extraction of lichens with acetone-alcohol or hot acetone, concentration of and crystallization from the extract[3,7,8,9]; yield from *Ramalina reticulata* 0.17%,[9] *R. capitata* 0.60%, *Usnea dasypoga* 1.15%, *U. florida* 1.80%, *U. hirta* 1.50%.[7,8]

CHEMISTRY

Constitution. $C_{18}H_{16}O_7$[2,3,4,7,10,11]; *l*- and *d*-stereoisomers; proposed structure[10,11]:



[For study of structure and derivatives, see ref. (11)].

Properties. Yellow rhombic crystals or needles; m.p. 204° C,[2] 193–194° C,[3] 202° C [5]; sublimes at 205–223° C at 5–8 mm; insoluble in water and glycerol; slightly soluble in methanol, ethanol, isopropanol, petroleum ether; dissolves with heat in camphor, phenol, paraffin, aniline, benzene, toluene, xylene, chloroform, ethylene trichloride, carbon tetrachloride, dioxan and glycols; dissolves in sodium hydroxide and sodium bicarbonate [5]; $[\alpha]_D^{25} = +495°$,[10] $+498°$ [3]; $[\alpha]_D^{20} = -452°$ [5]; $[\alpha]_D = 492°$ (chloroform) [2]; ultraviolet maxima at 226–230, 284 mμ,[3] $E^{1cm}_{0.0166\,mg/ml} = 1.167$ (284 mμ in chloroform).[8]

Reactions. See references [2, 10, 11].

BACTERIOLOGY

Assay. Cylinder-plate with *B. subtilis.*[1]

Spectrum in vitro. At 50 γ/ml, inhibition of *K. pneumoniae, E. coli, Pr. vulgaris, Ps. aeruginosa, S. aerotrycke, S. typhi-murium, Shigella, Staph. aureus, M. tuberculosis bovis* and *avis*, and soil mycobacteria; pneumococcus inhibited at 10–20 γ/ml, *St. hemolyticus* at 10–50 γ/ml and *M. tuberculosis hominis* at 20–50 γ/ml.[9]

Inhibition dilution (× 10,000) [7]:

Myco. tuberc. hom.	8–100	*Staph. aureus*	10–25
Myco. tuberc. bov.	64	*St. pyogenes*	12.5 –16
Myco. tuberc. av.	32	*E. coli*	<0.25
Myco. phlei	20	*Eb. typhi*	<0.25
Myco. smegmatis	20–25	*Pen. notatum*	<0.25

The *d*- and *l*-usnic acids have similar antibiotic activity.[7]

Factors affecting activity. No antagonism by serum.[7]

Mode of action. Bactericidal on pneumococcus, *St. hemolyticus* and most strains of *Myco. tuberculosis hominis.*[7]

Spectrum in vivo. In guinea pigs, experimentally infected with tuberculosis, treated subcutaneously with 10–20 mg/day of usnic acid in "Tween-80"-sesame oil [9]:

	No. animals	Mortality 0–32 days
Untreated	4	75%
"Tween-80"-sesame oil	10	40%
Usnic acid-"Tween-80"-sesame oil	10	20%

In guinea pigs with experimental tuberculosis infections, 15 mg/day of the sodium salt of usnic acid, administered orally, resulted in some improvement of treated animals as compared with controls.[4]

In humans, treatment of tuberculosis with usnic acid showed inconclusive results in preliminary trials.[4]

PHARMACOLOGY

Toxicity. In mice, subcutaneously, 1.5–2.0 mg was lethal; in guinea pigs, 30 mg was lethal, 15–20 mg daily was tolerated.[4, 9]

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USTIN

First observed by Kurung in 1945[1]; separated into two crystalline and one semi-crystalline fractions by Hogeboom and Craig in 1946[2]; the carbonate soluble predominant fraction named USTIN by Doering, Dubos, Noyce and Dreyfus in 1946[3]; derived from *Aspergillus ustus*.

PRODUCTION

Fermentation. Surface growth on Czapek-Dox medium with 4% glucose, 0.1% yeast extract for 14–16 days at 28°; initial pH 5.8, final pH 8.0–8.4.[1, 2]

Isolation. (A) Culture liquor extracted with ether, chloroform, or acetone; or by adsorption of activity on activated carbon, and elution with ether at pH 8.0–8.4; evaporated to a light yellow, amorphous residue.[1]

(B) Culture liquor extracted with ether; ether extract evaporated in vacuo, yellow gummy residue dissolved in phosphate buffer at pH 11.0; acidification resulted in light yellow precipitate; precipitate washed with water and dried in vacuo.[2]

(C) Culture liquor or mycelium extracted with ether.[3]

Purification. (A) By countercurrent distribution and recrystallization from ether-cyclohexane.[2]

(B) By fractionation into bicarbonate-soluble, carbonate-soluble and neutral portions; the carbonate-soluble fraction, ustin, recrystallized from toluene, acetic acid, ether or ether-hexane.[3]

CHEMISTRY

Constitution. Crystalline ustin by analysis C 53.42, H 3.69, Cl 24.27; neutralization equivalent 214, proposed $C_{19}H_{15}O_5Cl_3$.[3]

Fractions obtained by countercurrent distribution: I. Proposed $C_{21}H_{17}Cl_3O_6$, by analysis C 53.37, H 3.64, Cl 22.63; M.W. 350 (camphor); neutralization equivalent 250. II. Proposed $C_{21}H_{18}Cl_2O_6$; M.W. 330 (camphor).[2]

Properties. Crystalline ustin m.p. 184–6° C.[3] Fractions from countercurrent distribution: I. Heavy square plates m.p. 185–7° C (hot stage), ultraviolet maximum at 3,250 A. II. Rosettes of fine needles m.p. 214–6°, ultraviolet spectrum similar to previous.[2]

Insoluble in water, 0.1*N* HCl, soluble in 95% alcohol, 1% sodium carbonate, 0.1*N* NaOH, ethanol, acetone, ether, benzol; less soluble in cyclohexane; thermostable; inactivated by heat in alkali.[1, 2]

Reactions. Monomethyl, dimethyl and acetyl derivatives.[3]

BACTERIOLOGY

Assay. Serial dilution with *Myco. tuberculosis.*[1]

Spectrum in vitro. Inhibition of *Myco. tuberculosis* and *Myco. ranae* at 1:150,000–500,000; active against staphylococci and streptococci; inactive against *E. coli* and gram-negative bacteria; similar antibiotic activity by all *A. ustus* fractions.[1, 2, 3]

Factors affecting activity. Increased activity at lower pH; decreased activity by serum albumin, lipids, components of complex media.[3]

PHARMACOLOGY

Crude ustin, 6–8 mg intraperitoneally tolerated by mice.[1]

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VIOLACEIN

Antiprotozoal activity first observed by Singh in 1942[1,2]; antibacterial activity first described by Lichstein and Van de Sand in 1945[3]; derived from *Chromobacterium violaceum.*

PRODUCTION

Fermentation. Surface growth on medium containing g/l peptone 5, lactose 5, meat extract 3; incubated at 22° C for 14 days.[4]

Isolation. Bacterial cells separated by centrifuging, and extracted with acetone; acetone extract concentrated in vacuo, cooled overnight precipitating a nearly black, partly crystalline mass; precipitate filtered and dried in vacuo; yield 14 mg/l.[4]

Purification. Crude violacein extracted in a Soxhlet with dry chloroform followed by dry ether; extracts discarded; residue dissolved in boiling pyridine, filtered, filtrate concentrated and diluted with hot chloroform, and cooled; the precipitate washed with chloroform-pyridine followed by chloroform, dried in vacuo over P_2O_5; yield 78% of crude starting material.[4]

CHEMISTRY

Constitution. Proposed $C_{42}H_{35}O_6N_5$.[6]

Properties. Violet-black microcrystals, thick needles and elongated rectangular crystals; decomposes without melting upon heating[4]; slightly soluble in ethanol giving a deep violet solution, and in dioxan with a purple-red color.[3]

BACTERIOLOGY

Spectrum in vitro. Inhibition concentration (per cent)[3]:

Staph. aureus	0.002	*B. megatherium*	0.002
Staph. albus	0.002	*Cl. welchii*	>0.01
St. hemolyticus	0.001	*E. coli*	>0.03
St. viridans	0.001	*Eb. typhi*	>0.03
St. anhemolyticus	0.001	*Pr. vulgaris*	>0.03
Pneumococcus	0.003	*Ser. marcescens*	>0.03
C. diphtheriae	0.005	*N. intracellularis*	0.0005
B. subtilis	0.0005	*N. catarrhalis*	>0.01
B. anthracis	0.002	*H. pertussis*	>0.01
B. mesentericus	0.002	*V. comma*	>0.01

Br. abortus	>0.01
Sacch. cerevisiae	<0.0005
Bl. dermatitidis	0.001
Ep. floccosum	0.01
Trich. rubrum	0.01
Pen. notatum	>0.01

Violacein inhibited the ingestion of food by soil amebae, followed by death or encystment of the amebae; flagellates and ciliates were similarly affected.[1, 2, 6]

Mode of action. Bactericidal to *Staph. aureus* at 0.001–0.01% concentration.[5]

Factors affecting activity. Decreased activity by 10% sheep serum.[3]

Spectrum in vivo. In mice, no protection was afforded by 1–2 mg violaceum against an overwhelmingly infective dose of pneumococcus type II; against a lesser infective dose, a delayed death rate was indicated.[5]

PHARMACOLOGY

Toxicity. In mice, 1–2 mg intraperitoneally was tolerated. With guinea-pig granulocytes, no antiphagocytic activity was observed by 0.05–0.5 mg.[5]

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VIRIDIN

First described by Brian and McGowan in 1945[1]; derived from *Trichoderma viride.*

PRODUCTION

Fermentation. Surface growth on Czapek-Dox or Raulin-Thom medium with ammonium ion as nitrogen source; incubated at 25° C for 4–6 days; yield 2,000 or more *Botrytis allii* units or 45 mg/ml.[1, 2]

Isolation. Culture filtrate extracted with chloroform, the chloroform extract evaporated *in vacuo.*[1, 2]

Purification. Recrystallized from ethanol, methanol or benzene; yield 15 mg/l.[1, 2]

CHEMISTRY

Constitution. Proposed $C_{20}H_{16}O_6$, C 68.2, C 4.6, one OCH_3 8.8, two active H 0.6, one CH_3 attached to C 4.3, M.W. 352; found C 68.3, H 4.7, OCH_3 8.9, active H 0.5, CH_3 attached to C 3.5, M.W. 331 (bromoform).[1, 2]

Properties. Colorless rod-like prisms; m.p. 217–223° C (dec.); soluble in chloroform, bromoform; slightly soluble in carbon disulfide, carbon tetrachloride; almost insoluble in ether, camphor; $[\alpha]_D^{19} = -222°$ (1.0% in chloroform).[1, 2]

Reactions. Stable in aqueous solution at pH 2.9–3.8, less stable in neutral or alkaline solution; no color with ferric chloride; deep red-violet with phloroglucinol and HCl; reacts with hydrazine, phenylhydrazine and 2,4-dinitrophenylhydrazine; reduced over Raney nickel.[1, 2]

BACTERIOLOGY

Assay. Inhibition of spore germination of conidia of *Botrytis allii;* one unit equals 0.005 γ/ml.[1]

Spectrum in vitro. Antibacterial activity negligible; fungicidal to *Botrytis allii* and *Stemphylium* sp. at 10–30 γ/ml.[2]

Concentration for spore germination inhibition (γ/ml) [2]:

Aspergillus niger	3.1	*Penicillium digitatum*	0.2
Botrytis allii	0.005	*Penicillium expansum*	6.25
Cephalosporium sp.	0.8	*Penicillium notatum*	0.2
Cladosporium herbarum	0.2	*Stachobotrys atra*	6.25
Colletotrichum lini	0.003	*Stemphylium* sp.	0.2
Fusarium caeruleum	0.003	*Trichoderma viride*	>50
Fusarium culmorum	0.2	*Trichothecium roseum*	0.05

Factors affecting activity. Inactivated by peptone, yeast extract.[2]

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X 45

First described by Lehr and Berger [1]; probably identical with actinomycin [2]; derived from *Actinomycete* sp.

CHEMISTRY

Constitution. Proposed $C_{41}H_{54}O_{12}N_8$; by analysis C 58.0, 57.8, 58.1, H 6.5, 6.4, 6.4, N 13.1, 12.9, 13.1; contains threonine, proline, valine and N-methylvaline in association with a quinone system.[2]

Properties. Bright red crystals, decomposes at 252° C; $[\alpha]_2^D = -332°$ ($c = 0.25$, ethanol); infrared and ultraviolet absorption spectrum indistinguishable from that of actinomycin.[2]

BACTERIOLOGY-PHARMACOLOGY

Bacterial spectrum and toxicity indistinguishable from that of actinomycin.[2]

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XANTHOMYCIN

First described by Thorne and Peterson in 1948; derived from *Streptomyces* sp.; includes xanthomycin A and B.

PRODUCTION

Fermentation. Submerged culture (A) in shaker flasks, culture medium containing corn-steep liquor solids 1%, soybean meal 1%, dextrin 1%, K_2HPO_4 1%, NaCl 1%, $CaCO_3$ 0.1%; incubated at 25° C for 4–5 days, yield 5,000–8,000 *Staph. aureus* units per ml, up to 20,400 units/ml by selected strains with addition of lactose 0.5% to medium; yield drops sharply after 5 days. (B) In 30l fermenter with above medium, octadecanol in lard oil antifoam, inoculated with 4–6% vegetative growth in shaker flasks, air volume per minute to culture medium ratio 0.7, rpm 400, yield 20,000 units/ml with selected strain at 40 hours' incubation; yield drops sharply after 40 hours.

The original streptomyces strain was sensitive to its own antibiotic. By selection with xanthomycin added to the culture medium, improved strains were obtained.

Isolation. Culture filtrate treated with activated carbon; carbon cake washed with water saturated with butanol; activity eluted with 0.1*N* HCl saturated with butanol, eluate concentrated in vacuo, neutralized with NaOH, filtered to remove inert material; extracted with chloroform, activity removed from the chloroform extract with water at pH 2.0 or less.

Purification. Precipitation with picric acid or ammonium reineckate, followed by countercurrent distribution of the hydrochloride, yielding xanthomycin A 65–90%, B 10–35%, and about 1% of an unknown antibiotic. Xanthomycin A precipitated as the reineckate and recrystallized from 95% alcohol. Xanthomycin B reineckate not crystallizable. Regenerated by dissolving in methanol, decomposing with HCl; precipitate dissolved in anhydrous methanol, precipitated with ether, dried in vacuo.

CHEMISTRY

Constitution. Xanthomycin A reineckate: proposed $C_{38}H_{57}N_{12}O_{13}S_4Cr$, calculated: C 42.64 H 5.37 N 15.70 S 11.98; found: C 42.68 H 5.40

N 15.43 S 12.10; calculated equivalent weight and molecular weight by diffusion rate 742.

Properties. Xanthomycin A: yellow amorphous; (reineckate long orange needles m.p. 165–170° C (decomp.)); soluble in water, methanol, chloroform, butanol; less soluble in ethyl acetate, amyl acetate, ether; ultraviolet spectrum maxima at 254–267 mμ and 325–327 mμ; in chloroform-buffer systems, distribution coefficient increases with increase of pH.

Xanthomycin B has similar properties.

Reactions. Stable in acid solution, unstable above pH 6.0, negative Sakaguchi, Molisch, ninhydrin.

BACTERIOLOGY

Assay. Serial dilution and turbidimetric with *Staph. aureus.* Xanthomycin A picrate had a potency of 293,500 units/mg; the reineckate, 490,000 units/mg, the hydrochloride, 565,000 units/mg (calculated from reineckate, 670,000 units/mg, difference attributed to loss of activity on regeneration).

Spectrum in vitro. Inhibition dilution, xanthomycin A hydrochloride:

Staph. aureus	5.75×10^8
B. subtilis	1.53×10^8
E. coli	3.0×10^6
S. marcescens	6.0×10^6

Inhibition dilution, xanthomycin picrate:

Staph. aureus	3.0×10^8	*B. mesentericus*	4.0×10^6
Staph. albus	2.4×10^9	*B. megatherium*	4.0×10^6
Micro. subcitreus	2.4×10^8	*B. cereus*	2.4×10^6
Micro. flavescens	2.0×10^8	*B. niger*	2.0×10^8
E. coli	2.4×10^6	*B. brevis*	4.0×10^6
Pr. vulgaris	2.4×10^6	*B. albolactis*	4.0×10^5
Ser. marcescens	4.0×10^6	*B. graveolans*	4.0×10^5
Aero. aerogenes	4.0×10^6	*B. fusiformis*	8.0×10^7
S. gallinarum	2.4×10^6	*B. vulgatus*	2.0×10^7
Alcal. viscosus	4.0×10^7	*B. subniger*	4.0×10^7
Sar. lutea	4.0×10^7	*Myco. tuberc. avian*	<2,000
B. subtilis	2.4×10^8	*Myco. tuberc. BCG*	<2,000
B. mycoides	2.8×10^7	*Myco. tuberc. TB-1*	<2,000

Xanthomycin A and B picrates have similar bacterial spectra.

PHARMACOLOGY

LD50 of xanthomycin A hydrochloride administered intravenously to mice was approximately 3.2 γ (1,808 μ). The purest xanthomycin B preparation was equally toxic.

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XG

First described by Lewis, Hopper and Shultz in 1946[1]; derived from *Bacillus* sp. closely related to *B. subtilis*.

PRODUCTION

Fermentation. Surface growth on Czapek-Dox medium with 1% yeast extract; on peptone medium with 1% yeast extract; on peptone medium.[1]

Isolation. Culture filtrate activity adsorbed on activated carbon at pH 7.0, eluted with aqueous acetone.[2]

CHEMISTRY

Constitution. Amphoteric polypeptide with several different amino acids.[2]

Properties. Soluble in methanol and ethanol; insoluble in ether, chloroform, ethyl acetate, dry acetone; thermostable in culture liquor at pH 3.0 and 7.0; stable in wet acetone, anhydrous alcohol; forms a gel with water which is biologically inactive, reactivated by dissolving in anhydrous solvents.[2]

BACTERIOLOGY

Assay. Serial dilution with *Trichophyton gypseum;* purified XG 250 units/mg.[2]

Spectrum in vitro. A number of bacterial species and strains were largely completely resistant.

Fungi, inhibition concentration (units/ml):

Sporotrichum schenkii	11.2
Microsporum canis	0.7
Trichophyton rubrum	11.2
Cryptococcus neoformans	1.4
Trichophyton gypseum	2.8
Epidermophyton floccosum	2.8
Microsporum audouini	11.2
Rhodotorula	1.4
Monilia albicans	1.0

Factors affecting activity. No effect on activity by rabbit serum; activity reduced by incubation at lower temperatures, by increased number of organisms; activity increased with rate of growth of organisms.[2]

Mode of action. Fungistatic to fungicidal.[2]

PHARMACOLOGY

Toxicity. LD50 for mice with XG (100 units/mg) administered intravenously, 9,900 units/kg aqueous, 16,500 units/kg in saline; no toxicity by 4,100 units/kg twice daily for 5 days.[2]

In rats, 30,000 units/kg subcutaneously was nontoxic.[2] In rabbits, 5,000–10,000 units/kg intravenously was instantly lethal; mortality lowered by use of saline as diluent; no toxicity by 500–1,000 units/kg; 2,000–5,000 units/kg intramuscular or oral were tolerated.[2]

XG was strongly hemolytic in vitro.[2]

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GLOSSARY

Antagonism. The counteracting or neutralizing effect of certain substances, *e.g.*, cysteine, on the action of certain antibiotics; not necessarily an irreversible inactivation of the antibiotic.

Assay. The procedure for estimating the concentration of an antibiotic. Assay methods (other than chemical and physical antibiotic assay methods) may be classified as direct or indirect and the assay results described as absolute or relative. The direct assay method relates the concentration of antibiotic directly to the growth inhibition (or less commonly the death) of the test organism, whereas in indirect methods other criteria (*e.g.*, acid production, luminescence) are utilized and the relation of these criteria to growth inhibition is either inferred or preferably demonstrated. Absolute assay results are obtained by the dilution and to some extent by the turbidimetric method, *i.e.*, the antibiotic concentration can be expressed definitely in micrograms or units per ml as derived from the endpoint at which growth inhibition of the test organism occurs, and needs no prior-established relation or calibration against a standard. (In practice, however, random fluctuations of endpoint are accepted as routine and standard antibiotic solutions are commonly assayed simultaneously with unknown antibiotic solutions). Relative assay results are obtained by the diffusion and other assay methods. In the diffusion method, for example, the assay result is obtained as a diameter of the zone of inhibition, usually expressed in millimeters and meaningless unless related to the corresponding diameter of a known antibiotic solution.

The most commonly-used procedures (other than chemical and physical antibiotic assay methods) are:

(1) *Acid production.* Antibiotic activity measured by suppression of acid production by a suitable acid-forming test organism.

(2) *Agar-plaque.* A diffusion method. A plug ("plaque") of nutrient agar is removed, *e.g.*, by a sterile cork borer, from a petri dish on which the antibiotic-producer has been inoculated and incubated, and the plug placed on nutrient agar seeded with the test organism; after incubation, antibiotic activity is measured by the diameter of the zone of growth inhibition surrounding the plug.

(3) *Agar-spot.* A dilution method. Melted nutrient agar is seeded with a test organism; increments of antibiotic are added to the melted agar, and between increments, aliquots of melted agar are removed and "dropped" on a sterile petri dish; after incubation of the petri dishes, the minimal

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concentration causing growth inhibition is obtained by observing the concentration of antibiotic in the drop, or "spot," in which the test organism failed to grow.

(4) *Agar-streak plate.* A dilution method. A series of antibiotic concentrations are added to nutrient agar in a series of petri dishes; one or more test organisms are streaked over each agar surface and the plates incubated; the minimal growth inhibitory concentration of the antibiotic is observed for each test organism.

(5) *Agar-streak slant.* Similar to the agar-streak plate, but using agar slants.

(6) *Agar-well.* A diffusion method. From nutrient agar seeded with the test organism, discs of agar are removed, *e.g.*, by a sterile cork-borer, and the antibiotic solution poured into the "well"; after incubation, the antibiotic concentration is calculated from the diameter of the zone of inhibition as compared with a standard antibiotic solution.

(7) *Animal protection.* Antibiotic activity measured by ability to prevent death in laboratory animals, *e.g.*, mice, experimentally infected with a fixed dose of test organism.

(8) *Antiluminescence.* Antibiotic activity measured by inhibition of luminescence of the test organism, *e.g.*, *Photobacterium fischeri.*

(9) "*Curling*" — see "Stunting."

(10) *Cylinder-plate.* A diffusion method. On nutrient agar plates seeded with the test organism are placed small cylinders (glass, porcelain or stainless steel) which are filled with the antibiotic solution. To assure a uniformly level agar layer, either flat-bottomed petri dishes are used or a primary layer of unseeded agar is poured into the plate and, after hardening, overlaid with a secondary layer of seeded agar. After incubation, the diameter of the zones of inhibition is measured and compared with a standard antibiotic solution. In many instances, the diameter of the zone of inhibition is a logarithmic function of the antibiotic activity and when plotted on semilogarithmic paper gives a straight line over a considerable range of zone diameters. Within this range, the concentration of the unknown antibiotic solution is obtained by comparison with a standard, either graphically by means of a standard curve or by a mathematical computation.

(11) *Oxygen consumption.* Antibiotic activity measured by the inhibition of respiration in test organisms.

(12) *Paper-disk plate.* Similar to the cylinder-plate method but using filter paper disks in place of cylinders; a measured volume of antibiotic solution is added to each disk, or the disk is soaked in the antibiotic solution.

(13) *Hemolysis.* Antibiotic activity measured either by hemolysis of red blood cells by the antibiotic (applicable only if the antibiotic has hemo-

lytic properties) or inhibition of hemolytic activity by a test organism, *e.g.*, hemolytic streptococcus.

(14) *Serial dilution.* The minimal concentration causing growth inhibition is obtained by the addition of the antibiotic in graded concentrations to nutrient broth tubes inoculated with the test organism; after incubation, the minimal inhibitory concentration is measured by interpolation between the concentration causing growth inhibition and the next higher dilution allowing growth.

(15) *Spore germination.* A dilution method. Antibiotic activity measured by inhibition of the germination of spores of a suitable test organism, usually a fungus.

(16) *"Stunting" or "curling."* A dilution method. Antibiotic activity measured by degree of distortion of the growing hyphae of fungus mycelium.

(17) *Turbidimetric.* Antibiotic activity measured by inhibition of growth in liquid media, the extent of growth determined by the density or turbidity as indicated on a colorimeter or other means of measuring optical transmission.

Bactericidal. The lethal action of antibiotics on bacteria, as contrasted to bacteriostasis or growth inhibition. Recent evidence has tended to minimize the distinction between bactericidal and bacteriostatic activity. The principle variable factor is the time of exposure, *i.e.*, a bacteriostatic antibiotic is usually increasingly lethal as the exposure time is increased, and conversely a bactericidal antibiotic may be merely growth inhibitory under conditions of a short exposure time.

Bacteriostatic. The growth inhibitory action of antibiotics on bacteria — cf. **Bactericidal.**

Blood level. Concentration of antibiotic in the blood stream after administration, usually assayed as found in the plasma or serum.

CD50. Curative Dose 50%, a measure of in vivo antibiotic activity, *i.e.*, the quantity of antibiotic which will protect 50% of the laboratory animals experimentally infected with a lethal microorganism.

Chromatography. A general method for isolating the components of a mixture by passage through a column of some suitable substrate, *e.g.*, activated aluminum oxide. For analytical separations, filter paper strips are employed.

Countercurrent distribution. A general method for isolating the components of a mixture by utilizing the differing partition coefficients of the members of the components in a selected two-phase liquid system.

Culture medium. The nutrient solutions used for the growth of microorganisms may be classified as natural or synthetic. The natural culture media contain as general nutrients such natural products as: corn steep liquor, soybean meal, yeast extract, meat extract, distiller's residues, etc. Synthetic media are composed of relatively pure chemical compounds as the source of carbon, of nitrogen and the other elements essential for growth. The best known of the synthetic media are:

Czapek-Dox g/l	
sucrose	30
$NaNO_3$	2–3
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
$FeSO_4 \cdot 7H_2O$	0.01

Raulin-Thom g/l	
sucrose	47
ammonium nitrate	2.7
tartaric acid	2.7
ammonium phosphate	0.4
potassium carbonate	0.4
magnesium carbonate	0.27
ammonium sulfate	0.16
zinc sulfate	0.047
ferrous sulfate	0.047
potassium silicate	0.047

Fungicidal. *Lethal action of antibiotics on fungi* — *cf.* **Bactericidal.**

Fungistatic. Growth inhibition of fungi by antibiotics — cf. **Bacteriostatic.**

Inactivation. Decomposition of an antibiotic, cf. **Antagonism.**

Inhibition concentration. The minimal concentration of an antibiotic, usually expressed in micrograms or units per ml, which will inhibit the growth of a test organism under standardized conditions.

Inhibition dilution. The inhibition concentration of an antibiotic, usually expressed in terms of dilution of an initial antibiotic solution; utilized for convenience or when the initial antibiotic concentration is not known. A known or arbitrary concentration of the antibiotic is serially diluted, *e.g.*, in steps of one-half, or one-tenth, etc., and the dilution added to the culture medium inoculated with the test organism. After incubation, the minimal concentration, *i.e.*, the endpoint, causing growth inhibition is expressed in terms of dilution of the intial antibiotic solution, *e.g.*, as one-ten-thousandth or 1:10,000. Where the initial antibiotic concentration is the solid antibiotic itself, the dilution may be converted to micrograms per ml, *e.g.*, 1:10,000 is equivalent to 100 γ/ml.

LD50. Lethal Dose 50%, a measure of toxicity, *i.e.*, the quantity of antibiotic, preferably expressed as milligrams per kilogram of animal or mg/kg, which when administered to experimental animals, will be 50% fatal. Toxicity may also be expressed as LD80, LD90, etc.

MLD. Minimal Lethal Dose, a measure of toxicity, *i.e.*, the minimal quantity of antibiotic, which when administered to experimental animals, will invariably cause death. The use of the MLD as a measure of toxicity has been largely replaced by the more precise LD50.

Phytotoxicity. Injurious or lethal effect of antibiotics on plants, corresponding to **Toxicity** in animals.

Potency. The concentration of antibiotic in a solid or liquid as measured in units by bioassay.

Renal Clearance. The rate at which an antibiotic is removed from the blood stream by the kidney and passed into the urine.

Resistance. The immunity of certain microorganisms to the action of relatively large concentrations of specific antibiotics. Resistance may be classified as:

(1) *Natural.* Characteristic of genera, species or varieties, without a history of previous exposure to the antibiotic.

(2) *Acquired or Induced.* In certain microorganisms, a previous exposure to sublethal concentrations of specific antibiotics results in their ability to tolerate increasing amounts of the antibiotic, frequently to concentrations several times the previously lethal concentration.

Cross or homologous resistance occurs when a microorganism susceptible to two or more different antibiotics, upon acquiring resistance to one by sublethal exposure, becomes equally resistant to the others. Although a cross resistance relation between two antibiotics does not necessarily indicate that they are identical, it does mean that their mode of action is very similar. On the other hand, the lack of cross resistance between two antibiotics indicates strongly that they are dissimilar.

Rickettsicidal. Lethal action of antibiotics on members of the *Rickettsia* — cf. **Bactericidal.**

Serum binding. The combination of an antibiotic with the components of serum.

Shaker flasks. A laboratory method for fermentation by submerged growth. The antibiotic producer is inoculated into culture medium in an Erlenmeyer flask and the fermentation allowed to proceed in a device which continuously shakes or swirls the culture media. The shaker flask simulates fermentation in a stirred aerated tank fermenter; variations in aeration rate are provided by varying the ratio between volume of culture medium and volume of Erlenmeyer.

Spectrum. The characteristic activity of an antibiotic, preferably expressed quantitatively in micrograms or units per ml, against a series of species of microorganisms.

Submerged growth. The method of fermentation in which the organism is allowed to propagate throughout the depth of the culture medium, usually accompanied by continuous agitation and, in the case of aerobic species, by continuous aeration, *e.g.*, in shaker flasks, in stirred or rotatory tanks.

Surface growth. The method of fermentation in which the organism is allowed to grow as a pellicle or mat on the surface of undisturbed culture medium in flasks, bottles or trays.

Synergism. The enhancement of the activity of one antibiotic by the activity of another so that the combined effect is greater than the sum of the activity of the two separately. For example, if the separate inhibition concentrations against a particular microorganism of antibiotic A and antibiotic B are 1.0 γ/ml and 2.0 γ/ml respectively, then in a solution containing 0.5 γ/ml of A, the addition of 1.0 γ/ml of B would provide the combined inhibition concentration in the absence of any synergism. However, if it were observed that in a solution containing 0.5 γ/ml of A, the addition of only 0.8 γ/ml of B sufficed to provide the combined inhibition concentration, then A at 0.5 γ/ml is 25% synergistic toward B. In similar fashion, the synergism of B toward A, and at varying concentrations of each may be determined.

Titer. The antibiotic concentration of a solution, usually in reference to assay by serial dilution.

Toxicity. Injurious or lethal effect of antibiotics upon animals, as produced by the administration of specific concentrations in units or mg, preferably related to the weight of the animal, *e.g.*, units per kg, to the dosage schedule, *e.g.*, single injection, twice daily, etc., to the form of the antibiotic, *e.g.*, the sodium salt, to the vehicle, *e.g.*, in normal saline, and to the site of administration, *e.g.*, intraperitoneally. Toxicity with reference to the effect of antibiotics may be briefly classified as:

(a) *Acute.* Causing death of the experimental animal immediately or within a short period of time, *e.g.*, 1–3 days; measured quantitatively by the LD50, MLD, etc.

(b) *Chronic or delayed.* Causing death or pathology several days or weeks after administration or following repeated sublethal doses.

(c) *Pathologic.* Not necessarily lethal, but adversely affecting body function, *e.g.*, kidney, liver, central nervous system, with corresponding abnormal histology upon post mortem.

(d) *Allergic.* Sensitivities, anaphylaxis, skin reactions, cardio-vascular manifestations, neurologic symptoms, etc.

Unit. The minimum concentration of an antibiotic, as measured by serial dilution method, which is inhibitory to the growth of a test organism under rigidly controlled conditions; expressed as units per ml of nutrient solution. The unit is probably the most fundamental characteristic of an antibiotic. When the total weight of solid per ml is known, it is readily converted into the convenient units per mg, a measure of potency. In antibiotics, which are available in the pure form the unit may be replaced to advantage by the weight of the antibiotic in micrograms. (In the case of streptomycin, one *E. coli* unit is equivalent to one microgram of free streptomycin base.) Where the chemical structure of the antibiotic is not known and pure material is not available, the unit is still useful in terms of an arbitrary standard, even though unrelated to the weight of pure antibiotic.

Viruscidal. Lethal action of antibiotics on members of the viruses — cf. **Bactericidal.**

ANTIBIOTIC-PRODUCING ORGANISMS

Organism	Antibiotic formed
Acetobacter sp.	kojic acid
Acrostalagmus cinnabarinus — see *Verticillium cinnabarinum*	
Actinomyces — see *Streptomyces*	
Actinomycetales sp.	mycomycin
Agaricus nebularis	nebularine
Alectoria sp.	usnic acid
Allium sativum	allicin
Alternaria porri	alternaric acid
Alternaria solani	
Anemone pratensis	anemonin, protoanemonin
Anemone pulsatilla	
Aspergillaceae sp.	mycocidin
Aspergillus albus	kojic acid
Aspergillus awamori	
Aspergillus candidus	candidulin, kojic acid
Aspergillus clavatus	clavacin, kojic acid
Aspergillus effusus	kojic acid
Aspergillus flavipes	penicillin
Aspergillus flavus	aspergillic acid, hydroxyaspergillic acid, kojic acid, penicillin
Aspergillus fumigatus	fumigacin, fumigatin, gliotoxin, kojic acid, spinulosin
Aspergillus giganteus	clavacin, kojic acid, penicillin
Aspergillus glaucus	kojic acid
Aspergillus gymnosardae	
Aspergillus luteo-virescens	
Aspergillus lutescens	
Aspergillus melleus	penicillic acid
Aspergillus nidulans	kojic acid, penicillin
Aspergillus niger	penicillin
Aspergillus ochraceus	penicillic acid
Aspergillus oryzae	kojic acid, penicillin
Aspergillus parasiticus	
Aspergillus quercinus	penicillic acid
Aspergillus sp.	citrinin
Aspergillus sulphureus	penicillic acid
Aspergillus tamarii	kojic acid
Aspergillus terreus	citrinin, clavacin, geodin
Aspergillus ustus	ustin
Bacillus alvei	alvein
Bacillus brevis	tyrothricin, gramicidin S
Bacillus cereus	biocerin

Organism	Antibiotic formed
Bacillus circulans	circulin
Bacillus krzemieniewski	polypeptin
Bacillus laterosporus	laterosporin
Bacillus licheniformis	ayfivin, licheniformin
Bacillus polymyxa	polymyxin
Bacillus simplex	simplexin
Bacillus sp.	colistatin, XG
Bacillus subtilis	bacilipin, bacillin, bacillomycin, bacilysin, bacitracin, endosubtilysin, eumycin, mycosubtilin, subtenolin, subtilin, subtilysin
Basidiomycetes sp.	corticin, grisic acid, irpexin, obtusin, pleurin
Buellia canescens	diploicin
Cassia reticulata	cassic acid
Centenella asiatica	asiaticoside
Cetraria islandica	usnic acid
Chaetomium cochliodes	chetomin
Chaetomium elatum	
Chaetomium funiculum	
Chaetomium spirale	
Chlorella pyranoidosa	chlorellin
Chlorella vulgaris	
Chromobacterium iodinum	iodinin
Chromobacterium sp.	chlororaphin
Chromobacterium violaceum	violacein
Cladina sp.	usnic acid
Cladonia alpestris	
Cladonia cristatella	
Cladonia sylvatica	
Clitocybe gigantea	clitocybin
Crepis taraxacifolia	crepin
Crepis virens	
Crotolaria crispata	citrinin
Diatora sp.	usnic acid
Eleocharis tuberosa	puchiin
Escherichia coli	colicine
Evernia sp.	usnic acid
Fusarium avenaceum	avenacein
Fusarium fructigenin	fructigenin
Fusarium javanicum	javanicin
Fusarium lateritium	lateritiin I
Fusarium othoceras	enniatin
Fusarium sambucinum	sambucinin
Fusarium sciroi	enniatin
Fusarium sp.	lateritiin II
Gymnoascus sp.	clavacin
Haemotomma sp.	usnic acid
Humulus lupulus	humulon, lupulon
Hydrocotyle — see *Centenella*	
Inoloma traganum	inolomin

Organism	Antibiotic formed
Lactarius deliciosus	lactaroviolin
Lecanora esculenta	lecanorin A
Lecanora sordida	roccellic acid
Lecanora sp.	usnic acid
Lycopersicon pimpinellifolium	tomatine
Marasmius conigenus	marasmic acid
"Meredith's actinomycete"	musarin
Metarrhizium glutinosum	glutinosin
Micrococcus varians	micrococcin
Micromonospora sp.	micromonosporin
Moringa pterygosperma	pterygospermin
Mycobacterium tuberculosis	phthiocol
Nocardia coeliaca	nocardin
Nocardia cyanea	litmocidin
Nocardia gardneri	proactinomycin
Oidiodendron fuscum	fuscin
Penicillium aurantio-virens	puberulic acid, puberulonic acid
Penicillium brevi-compactum	mycophenolic acid
Penicillium chrysogenum-notatum	penicillin
Penicillium chrzaszzi	citrinin
Penicillium cinarescens	spinulosin
Penicillium citreo-roseum	penicillin
Penicillium citreo-sulfuratum	citrinin
Penicillium citrinum	
Penicillium claviforme	clavacin
Penicillium corylophylum	penatin
Penicillium crustosum	penicillin
Penicillium cyclopium	penicillic acid
Penicillium cyclopium-viridicatum	puberulic acid, puberulonic acid
Penicillium dalae	kojic acid
Penicillium divergens	clavacin, gentisyl alcohol
Penicillium expansum	citrinin, clavacin
Penicillium gladioli	gladiolic acid
Penicillium griseofulvum	griseofulvin, mycelianamide
Penicillium herquei	herquein
Penicillium implicatum	citrinin
Penicillium janczewski	griseofulvin
Penicillium jenseni	gliotoxin
Penicillium johannioli	puberulic acid, puberulonic acid
Penicillium leucopus	clavacin
Penicillium lividum	citrinin
Penicillium notatum	penatin, penicillin
Penicillium obscurum	gliotoxin
Penicillium patulum	clavacin, griseofulvin, gentisyl alcohol
Penicillium phoeniceum	phenicin
Penicillium phoeo-janthinellum	citrinin
Penicillium puberulum	penicillic acid, puberulic acid, puberulonic acid
Penicillium resticulosum	penatin
Penicillium rubrum	phenicin

Organism	Antibiotic formed
Penicillium sp.	clavacin, gentisyl alcohol, gliotoxin, penicidin
Penicillium spinulosum	spinulosin
Penicillium stoloniferum	mycophenolic acid
Penicillium suavolens	penicillic acid
Penicillium tardum	tardin
Penicillium thomii	penicillic acid
Penicillium urticae	clavacin, gentisyl alcohol quinhydrone
Parmelia sp.	usnic acid
Platysma sp.	
Pleurotus griseus	pleurotin
Plumbago europaea	plumbagin
Plumbago rosea	
Plumbago zeylonica	
Polyporus biformis	biformin, biforminic acid
Polystictus sanguineus	polyporin
Proactinomyces — see *Nocardia*	
Pseudomonas aeruginosa	pyo compounds, pyolipic acid
Ramalina reticulata	usnic acid
Ranunculus acer	anemonin
Ranunculus alpester	
Ranunculus arvensis	
Ranunculus bulbosus	anemonin, protoanemonin
Ranunculus hirsustus	protoanemonin
Ranunculus japonicum	anemonin
Ranunculus montanus	
Ranunculus sceleratus	
Raphanus sativus	raphanin
Serratia marcescens	prodigiosin
Squamaria sp.	usnic acid
Stereum hirsutum	hirsutic acid
Streptococcus sp.	diplococcin, nisin
Streptomyces albus	actinomycetin
Streptomyces antibioticus	actinomycin
Streptomyces aureofaciens	aureomycin
Streptomyces bikiniensis	streptomycin
Streptomyces fradiae	neomycin
Streptomyces griseus	actidione, grisein, streptocin, streptomycin
Streptomyces lavendulae	lavendulin, streptothricin
Streptomyces rimosus	terramycin
Streptomyces rochei	borrelidin
Streptomyces sp.	actimomycin, actinorhodine, actinorubin, antimycin, anti-smegmatis, EI5, streptin, streptolin, streptothricin, sulfactin, X45, xanthomycin
Tilachlidium sp.	cynematin
Trichoderma viride	gliotoxin, viridin
Trichothecium roseum	trichothecin
Usnea barbata	usnic acid
Verticillium cinnabarinum	chetomin

Author Index

Abazza, A., 224
Abraham, E. P., 47, 48, 68, 169, 204
Abrams, R., 260
Ackerman, H., 263
Ainsworth, G. C., 198
Alderton, G., 237, 238
Alderton, H. H., 158
Alexander, H. E., 90
Alfrey, V., 230
Alsberg, C. L., 167, 169, 182, 183
Alverson, C., 57
Alverson, C. M., 199
Ambrose, A. M., 99
Ananenko, N. H., 81, 82, 198
Andersen, A. A., 158
Anderson, D., 65
Anderson, H. H., 158, 238
Anderson, H. W., 85, 89, 104
Anderson, R. J., 188
Anderson, T. F., 180
Anigstein, L., 64
Anker, H., 72, 75
Anslow, W. K., 103, 121, 122, 123, 164, 165, 217, 219
Antopol, W., 76
Appleby, J. C., 258
Arnstein, H. V., 143, 145, 162, 164
Arriagada, A., 66, 68
Asahina, Y., 49, 205, 206
Atkinson, M., 181
Avery, R. G., 57, 58
Azarkh, R. M., 258

Bachman, M. C., 76
Baehr, H., 49, 206
Bailey, C. A., 91
Bailey, J. H., 43, 45, 133
Baker, H., 246
Baker, M. J., 199
Baker, W., 123
Baker, Z., 260
Ballantine, R., 232
Barber, T. E., 65
Barger, G., 208
Barker, N. W., 262
Barnes, B. W., 246
Barnes, E. M., 149
Barnes, T. G., 246
Baron, A. L., 258
Barry, G. T., 75, 265
Barry, V. C., 111, 112, 217
Barta, J., 104, 126
Bartz, Q. R., 85, 89
Bean, C. W., 261
Beaudette, F. R., 40
Becker, B. C., 49, 206
Beigelman, P. W., 64
Bekurts, E., 206
Bekurts, H., 50
Bell, P. H., 199
Bellows, J. G., 76
Belozersky, A. N., 259
Belton, F. C., 68
Benedict, R. G., 191, 198, 199
Beninson, J., 64
Benoist, D., 180
Benson, D. V., 76
Bergel, F., 103, 127
Berger, J., 79, 80, 269, 270
Bergstrom, S., 214
Berkman, S., 238
Bernheim, F., 66
Berridge, N. J., 175
Bhattacharyya, P. K., 85, 89
Bhattacharyya, S. C., 53
Birkinshaw, J. H., 104, 121, 124, 126, 169, 180, 183, 207, 208, 217, 219
Biro, B., 75
Bischoff, H., 91
Black, O. F., 167, 169, 182, 183
Blair, J. E., 104
Blanchon, E., 191
Blatchley, E. D., 68
Blinnikova, E. I., 258
Bliss, E. A., 63, 64, 90, 199, 246
Bloomfield, S., 262
Boas, F., 48, 49
Bohonos, N., 35, 37
Boiteau, P., 53
Boller, A., 114, 115
Bond, G. C., 76
Bone, J. F., 199
Bonet-Mawry, P., 191
Bordley, J. E., 260
Borodin, N., 241, 242
Bose, S. R., 201, 202

Brack, A., 126, 265
Bracken, A., 104, 121, 124, 126, 169, 219
Brainerd, H. D., 64
Braley, A. E., 64
Brande, A. I., 64
Brandon, B., 259
Brazhnikova, M. G., 155, 251, 258
Brewer, C. R., 231
Brian, P. W., 46, 47, 128, 132, 133, 134, 136, 137, 138, 249, 250, 268, 269
Brickhouse, R. L., 64
Brockmann, H., 41
Brodersen, R., 50, 110
Brook, M. J., 94
Broom, W. A., 104, 181
Broschard, R. W., 64, 198
Brousseau, D. Z., 231
Brown, A. M., 191, 198
Brown, H. B., 75
Brown, P. P., 100
Brownlee, G., 191, 198, 199
Bruce, W. F., 132, 133
Bruyn, H. B., 63
Bryan, C. S., 261
Bryer, M. S., 64, 65, 199, 246
Brzeski, W., 126
Bucca, M. A., 232, 234
Buck, J. S., 46
Buck, M., 80
Bugie, E., 85, 161, 162, 222, 231
Bulbring, E., 104
Burdon, K. L., 117, 167
Burger, A., 66
Burke, F. G., 91
Burkholder, P. R., 89, 263, 265
Burkholder, T. M., 75
Burton, H. S., 138, 139, 183, 187
Bush, M. T., 57, 58
Bushby, R. M., 199
Butlin, K. R., 162, 163
Buzas, A., 53
Bywater, W. G., 260

Cain, C. K., 180
Calam, C. T., 127
Caldwell, E. R., 246
Callow, R. K., 152, 154
Carle, B. N., 199
Carpenter, C. M., 263
Carr, J. G., 181
Carson, J. F., 158, 238
Carter, H. E., 85, 89, 104
Cartwright, N. J., 100
Castaneda, M. R., 64
Castle, H., 265
Catch, J. R., 198, 199
Cattaneo, C., 258
Cavallito, C. J., 43, 45, 46, 133
Cecil, R., 180
Chaffee, E., 259
Chain, E., 100, 103, 118, 121, 202, 204
Chandler, C. A., 63, 64, 199, 246
Chang, N., 158
Chang, S. M., 246
Chapman, P. J., 104
Chase, F. E., 104
Chen, S. L., 209
Cheng, B. L., 209
Cheng, W. K., 209
Chin, Y. C., 158, 238
Chinn, B. D., 90
Chittenden, G. E., 91
Chouteau, J., 191
Christensen, H. N., 259
Clapp, M. P., 263
Clark, C. F., 261
Clarke, H. T., 186
Clemo, G. R., 142, 143
Clift, M. E., 76
Clutterbuck, P. W., 127, 169
Coburn, A. F., 260
Colingsworth, D. R., 201, 230
Colio, L. G., 70, 71
Collins, H. S., 64
Collins, K., 76
Conn, J. B., 258
Conn, J. F., 85
Consden, R., 259
Controulis, J., 89
Cook, A. H., 114, 143, 145, 147, 148, 162, 164
Cook, M. A., 232
Cook, M. H., 198
Cooper, M., 259
Copley, M. J., 110
Cordon, T. C., 217
Coret, I. A., 76
Corson, M. E., 232
Couch, J. F., 110, 214, 215
Coulston, F., 261
Coulthard, C. E., 177, 180, 181
Cox, H. R., 64
Cox, S. F., 114, 147, 148
Coyle, J. E., 76
Coyne, F. P., 98
Craig, L. C., 75, 259, 265, 266
Cram, D. J., 99
Crocker, T. T., 90
Crooks, H. M., Jr., 89
Crowe, S. J., 260, 262
Crowfoot, D. M., 121
Cullen, M., 247
Curd, F. H., 265
Curnen, E. C., 262

Curtis, P. J., 46, 47, 128, 134, 136, 137, 138, 269

Dalgliesh, N. E., 270
Darker, G. D., 75
Datta, N. L., 46
Dauber, H. J., Jr., 104
Davide, H., 214
Davis, J. G., 143
DeBeer, C., 230
DeEds, F., 99
Denkelwater, R., 232
Dickison, H. L., 57
Dill, W. A., 90
Dimick, K. P., 237, 259
Dobbert, N. N., 258
Doering, W. E., 265, 266
Doisy, E. A., 121, 180
Dolowitz, D. A., 260
Donovick, R., 231, 232
Doolittle, S. P., 247, 249
Dorfman, A., 260
Dornbush, A. C., 64
Dorrer, O., 208
Dougherty, N., 273
Dowling, H. F., 64, 246
Downs, P. M., 260
Dreyfus, R., 265, 266
Dub, L., 260
Dubos, R. J., 65, 251, 257, 258, 260, 261, 265, 266
Duggar, B. M., 58, 63
Dunn, C. G., 147
Dunn, J. T., 191
Dunshee, B. R., 52
Dureuil, M., 53
Dutcher, J. D., 57, 132, 133

Eagle, H., 75, 76, 77, 238
Edwards, R. R., 259
Ehrlich, J., 85, 89, 90
Eisenberg, G. M., 191, 198
Eldridge, D., 80
Elliot, W. H., 121
Elvenjem, C. A., 232
Emerson, O. H., 132
Emerson, R. L., 35, 37
Emmart, E. W., 175
Enders, J. F., 262
Engel, B. G., 126
English, J. P., 199
Erichsen, L., 180
Ettlinger, L., 113, 114
Evans, A. W., 263, 265
Evans, F. L., 76
Evenson, A., 232
Ewart, A. J., 98

Fainshmidt, O. I., 165, 166
Farber, S. M., 238
Farkas, R., 64
Farmer, C. J., 76
Farmer, T. H., 114, 147, 148
Farr, A. C., 80
Faust, E. P., 64
Feeney, R. E., 237
Feldman, W. H., 232
Fellows, C. E., 199
Ferraro, W. R., 261
Feustel, I. C., 100, 234, 237, 259
Fevold, H. L., 237
Fieser, L. F., 191
Fildes, P., 66
Finland, M., 64, 246
Finlay, A. C., 242, 246
Fisher, A. M., 260
Fisken, R. A., 90
Fleischman, R., 76, 238
Fleming, A., 184, 186
Florey, H. W., 68, 100, 103, 104, 106, 107, 118, 121, 141, 169, 204, 241, 242
Flynn, E., 230
Foley, E. J., 260
Foley, M. K., 260
Folkers, K., 174, 230, 231
Fong, J., 91
Fontaine, T. D., 247, 249
Ford, J. H., 35, 37
Forscher, B. K., 199
Foster, J. W., 69, 104, 147, 183, 217, 220, 230, 231
Fraenkel-Conrat, H., 259
Fragner, P., 141
Francis, A. E., 262
Francis, T., Jr., 246
Frankel, J., 174
Fredericq, P., 106, 107
Free, A. H., 75
Freeman, G. G., 249, 250
Fried, J., 230
Friedemann, T. F., 147
Friedheim, E. A. H., 187
Friedman, R., 198
Fujita, A., 49, 205, 206
Fuller, F. W., 64
Fulton, C. O., 230

Gaeumann, E., 85, 113, 114
Gage, R. P., 263
Galbraith, M., 90
Gale, P., 174
Gardner, A. D 202, 204
Gardner, G. M., 112
Garibaldi, J. A., 237
Garson, W., 94, 201

Gauld, R. L., 90
Gause, G. F., 107, 108, 154, 155, 251, 258
Gautron, G., 106
Geiger, W. B., 40, 121, 123, 132, 161, 162, 220
George, M., 206
Gerber, I. E., 166
Gill-Carey, D., 183
Gilliver, K., 47, 48, 169
Glazko, A. J., 90, 91
Glister, G. A., 57
Glover, R. E., 154
Gocke, A. H., 246
Goldberg, J., 75
Goldberg, M. W., 79, 80
Goll, M., 99
Goodlow, R. J., 78, 79
Goodman, J. J., 239
Goodman, S. C., 75
Goorley, J. T., 75
Gordon, A. H., 258, 259
Gore, R. C., 199
Gore, T. S., 99, 100
Gorman, R. D., 238
Gosio, E. J., 167
Goth, A., 37, 57, 58, 110
Gottlieb, D., 85, 89
Gottshall, R. Y., 110
Graber, R. P., 230
Graessle, O. E., 174, 231, 232, 260
Grant, G. A., 230
Gratia, A., 106, 107
Green, R., 90
Greenberg, J., 261
Greer, H. A., 147
Gregory, J. D., 75, 259
Greif, R., 75
Gross, M., 166
Grove, J. F., 128, 138
Grubb, T. C., 104
Gruhzit, O. M., 90
Grunberg, E., 80
Gundel, M., 232
Guy, E. G., 90
Gye, W. E., 104

Hac, L. R., 261
Haenseler, C. M., 217
Hagikara, M., 104
Hall, W. H., 64
Hallman, F. A., 104
Hambly, A. S., 238
Hammond, C. W., 246
Hampton, J. W. F., 104
Hamre, D. M., 57, 231, 232
Hansen, E. L., 238
Harned, B. K., 63
Harris, D. A., 220, 221
Harris, G. C. M., 100, 103, 118, 121, 128, 143, 145, 147, 169, 241, 242
Harris, W. W., 65
Harrison, R. W., 260
Hart, A. B., 232
Hart, P. D., 152, 154
Hartree, E. F., 180, 181
Hassal, C. H., 234, 237
Hays, E. E., 213
Heatley, N. G., 68, 99, 104, 106, 107, 108, 109, 141, 208, 219
Hedström, H., 171
Hegsted, D. M., 259
Heilala, P., 265
Heilman, F. R., 65, 247, 260, 261, 262, 263
Heishman, S. O., 261
Hemming, H. G., 46, 47, 128, 132, 133, 134, 136, 137, 138, 249, 250, 269
Henderson, J., 261
Henle, G., 260
Henry, A. W., 239
Henry, R. J., 238
Herbst, E. J., 231
Herrell, W. E., 186, 224, 247, 260, 261, 262, 263
Herrick, J. A., 104
Herrick, H. T., 147
Hersell, W. E., 65
Hervey, A., 77, 78, 108, 138, 143, 158, 176, 188, 189, 190
Hetherington, A. C., 94, 98
Hill, H. H., 53, 57
Hill, L. M., 64
Hillegas, A. B., 90
Hills, G. M., 68, 154
Hinshaw, H. C., 232
Hirsch, A., 174, 175
Hirschhorn, H. N., 232, 234
Hirschman, D. J., 234, 237
Hirschy, H. W., 99
Hobby, G. L., 246, 259, 273
Hoffhine, C. E., Jr., 174, 230
Hogeboom, G. H., 265, 266
Holbrook, W. A., 91
Holden, M., 49, 206
Hollande, A. C., 105, 106
Holmes, A. M., 47, 48
Hoogerheide, J. C., 57, 121, 123, 132, 232, 251, 258
Hooper, I. R., 104
Hopkins, W. A., 104
Hopper, M. E., 272, 273
Horning, E. S., 83, 85, 100, 103, 104, 118, 121, 180, 225, 230
Horvath, S., 215, 216
Horwood, R. E., 261

Hotchkiss, R. D., 258, 260, 261
Housewright, R. D., 238
Howard, K. S., 199
Howell, S. F., 201, 234
Hubbard, W. N., Jr., 247
Huebner, R., 247
Humfeld, H., 100, 234, 237
Humphreys, E. M., 237
Hutchinson, D., 174

Ingalls, M. S., 232
Irving, G. W., Jr., 247, 249
Ivanovics, G., 215, 216

Jackson, E. B., 90, 246
Jackson, E. R., 199
Jackson, G. G., 246
Jampolsky, L. M., 80
Jann, G. J., 158, 238, 239
Jansen, E. F., 158, 234, 237, 238
Jennings, M. A., 68, 100, 103, 104, 118, 121, 141, 147, 169, 204
Johnson, B. A., 72, 75, 76
Johnson, C. W., 78, 79
Johnson, E. A., 117, 167
Johnson, F. G., 260
Johnson, H. C., 232
Johnson, J. R., 132, 133, 186
Johnston, F. R., 66
Joiris, E., 107
Jones, D., 40, 231
Jones, H., 260
Jones, H. L., 78, 79
Jones, H. M., 57
Jones, R. G., 37
Jones, T. S. G., 191, 198
Joslyn, A. A., 85, 89, 90
Judd, D. K., 262
Junowicz-Kocholaty, R., 40, 41, 43, 147, 150, 151, 230, 239, 240

Karow, E. O., 104, 147, 183
Kass, E. H., 246
Katzman, P. A., 104, 121, 180
Katznelson, J., 198, 217
Kaufmann, O. W., 94
Kavanagh, F., 77, 78, 82, 83, 99, 108, 138, 143, 158, 176, 188, 189, 190, 219, 231, 232
Keeble, C. F., 76
Keefer, C. S., 261
Keilin, D., 180, 181
Keitt, G. W., 50, 52
Kelly, D. R., 80
Kelner, A., 40, 41, 43, 52, 147, 150, 151, 239, 241
Kelso, R., 260
Kent, J., 104
Kidd, J. G., 133
Kimmig, J., 166
Kipping, F. B., 50, 206
Kirschbaum, A., 90
Kiser, J. S., 99, 208
Kissling, R. E., 176
Kjaer, A., 50, 110
Klein, M., 260
Klein, R., 103
Klose, A. A., 237, 238
Knelland, Y., Jr., 247
Knight, V., 91, 238, 246
Knowles, E., 258
Kocholaty, W., 40, 41, 43, 147, 150, 151, 177, 180, 230, 231, 239, 241
Koerber, W. L., 127. 232
Koffler, H., 94, 201
Kolbros, J. J., 232
Kolmer, J. A., 186, 224
Konikova, A. S., 258
Koreniako, A. I., 58, 165, 166
Kornfeld, E. C., 37
Krale, W. F., 262
Kramer, S. D., 147
Krassilnikov, N. A., 58
Krishna, M. C. R., 46
Krishnamurthi, A., 46
Kriss, A. E., 37
Krueger, A. P., 239
Krueger, R. A., 76
Krupka, G., 198
Kubsch, F., 265
Kuehl, F. A., Jr., 231
Kupferberg, A. B., 220, 221
Kurung, J. M., 265, 266

Lacey, M. S., 114, 143, 145, 147, 148, 162, 164
Laird, R. L., 64
Landerkin, G. B., 104
Landy, M., 70, 71
Langlykke, A. F., 191, 198
Larson, C. L., 65, 199
Laufer, S., 99
Lawrence, C. A., 262
Leach, B. E., 35, 37
Leben, C., 50, 52
Lechevalier, H. A., 171, 173, 174
Lederer, E., 53
Lee, S. W., 260
Lefler, J. S., 112
Lehr, H., 269, 270
Leidy, G., 90
Lembke, A., 166
Lenert, T. F., 246
Lennette, E. H., 64

Leon, A. P., 90
Lepper, M. H., 64, 246
Levaditi, C., 180, 224
Levin, F. S., 77
Lewis, G. M., 272, 273
Lewis, J. C., 158, 237, 238, 259
Ley, H. L., Jr., 90, 91, 246
Lichstein, H. C., 188, 204, 267, 268
Lightbody, H. D., 237
Lilligren, B. L., 90
Lindsay, J. R., 262
Lineweaver, H., 238
Lipman, F., 258
Lissovskaya, N. P., 251, 258
Lister, L. M., 90
Little, P. A., 64, 198
Little, R. B., 261
Lochhead, A. G., 104, 198
Loewe, H., 232
Loewenstein, J. S., 49, 206
Löfgren, N., 171
Logan, M. A., 64
Long, C. L., 78, 79
Long, L. M., 89
Long, P. H., 64, 65, 199, 246
Longacre, A. B., 76
Low, B. W., 121
Lowenthal, K., 104
Luckey, T. D., 232
Luteraan, P., 191
Lythgoe, B., 53

Ma, R., 249
Macht, D. I., 64
Maclay, W. D., 158
MacLean, I. W., 90
MacPhillamy, H. B., 57
Magoffin, R., 65
Manevich, A. A., 262
Mankikar, P. J., 90, 91
Mann, F. C., 263
Mann, G. E., 259
Mansberger, A. R., Jr., 246
Marcus, S., 127
Marin, E. D., 262
Marsh, W. S., 231
Marshak, A., 265
Marston, R. Q., 147, 204
Martin, A. J. P., 258, 259
Martin, F. E., 261, 262
Martino, E., 90
Mattick, A. T. R., 174, 175
May, O. E., 147
Mayer, R. L., 231
McAllister, R. C. A., 258
McCoy, E., 231, 232
McCrone, W. C., Jr., 132
McCullough, W. G., 231
McDermott, W., 91
McGowan, J. C., 128, 133, 134, 138, 268, 269
McIlwain, H., 92, 142, 143
McIntosh, J., 261
McKee, C. M., 57, 260
McLeod, C. M., 199, 201, 262
McNally, P. A., 217
McVay, L. V., 64
Meanwell, L. J., 111
Mecir, R., 104, 126
Mecklejohn, G., 64
Meleney, F. L., 72, 75, 76, 77
Menzel, A. E. O., 57, 121, 123, 132
Meredith, C. H., 164
Metzger, H. J., 40, 231
Meyer, E., 232
Meyer, K., 259
Michael, S. E., 104, 125
Michaelis, M., 99
Michaelis, R., 177, 180
Michener, H. D., 158, 169, 170
Miller, B. F., 260
Miller, C. P., 246
Miller, J. L., 76
Miller, L. L., 132
Miller, W. R., 232
Miller, W. T., 261
Mills, J. A., 58, 132
Mills, R. C., 231
Mirick, G. S., 262
Mizukara, Y., 104
Molitor, H., 232, 262
Mollari, M., 90
Moore, P. R., 232
Morgan, E. N., 169
Morris, M., 164
Morrison, A. L., 103, 127
Morrison, R. I., 249, 250
Morton, H., 41, 43, 52, 147, 150, 151, 241
Moss, A. R., 103
Most, H., 246
Moyer, A. J., 147
Mueller, J. H., 262
Mull, R. P., 132
Murnaghan, M. F., 183
Murray, F. J., 92, 94
Muskat, I. E., 49, 206
Musselman, A. D., 76, 238

Nager, V., 113, 114, 115, 116
Naghski, J., 110, 214, 215, 249
Neter, E., 104, 231, 263
Newman, E. V., 75, 77
Newman, M. S., 188
Newton, G. G. F., 68, 69, 71, 72

Nielsen, J. K., 90
Nook, M. A., 76
Noyce, D. S., 265, 266
Nungester, W. J., 76

Ogston, A. G., 180
Olcott, H. S., 259
Olive, T. R., 89
Olivier, H. R., 112, 113, 191
Olshaker, B., 91
Oppikofer, F., 46
Ordal, Z. L., 232
Ordanik, M., 158
Orr, W., 91
Osborne, E. M., 108, 109
Osborne, R. R., 90
Ott, W. L., 76
Oxford, A. E., 99, 110, 111, 123, 127, 136, 137, 147, 164, 165, 169, 183, 208, 220

Page, J. E., 99, 220
Paine, T. F., Jr., 64
Palmer, V. L., 261
P'an, S. Y., 247
Pandalai, K. M., 206
Pannell, L., 232
Panse, T. B., 99, 100
Parke, T. V. J., 37
Parker, A., 158
Parker, R. T., 247
Passhina, T. S., 259
Patiala, J., 265
Patiala, R., 265
Payne, H. G., 76
Pearson, J., 258
Peck, R. L., 174, 213, 230, 231, 260
Pelcak, E. J., 64
Pénau, H., 180
Perault, R., 180
Perlman, D., 231
Perry, T. L., 64
Petersen, E. M., 199
Peterson, D. H., 94, 201, 230
Peterson, W. H., 221, 222, 270, 271
Philip, C. B., 91
Philpot, F. J., 99, 104, 208, 219, 241, 242
Pickrell, K. L., 260
Pidacks, C., 63
Piersma, H. D., 259
Pincoffs, M. C., 90
Pini, H., 41
Pitskhelauri, G Z., 262
Plattner, P. A., 113, 114, 115, 116
Plotho, O. V., 41
Plotke, F., 65
Pollock, A. V., 99
Polonsky, J., 53
Pomerat, C. M., 263
Poole, J. B., 249
Porter, J. N., 198
Porter, W. L., 249
Portwood, L. M., 110
Posternak, T., 187
Pratt, R., 91
Preisser, W., 91
Prescott, S. C., 147
Price, C. W., 63
Pridham, T. G., 89
Prigot, A., 64
Puetzer, B., 104
Pugh, L. H., 231

Quilligan, J. J., Jr., 246
Quinn, L. Y., 94

Raby, W. T., 91
Raghunandana, R. R., 46
Raistrick, H., 94, 98, 99, 103, 104, 121, 122, 123, 124, 126, 127, 136, 137, 164, 165, 169, 180, 183, 207, 208, 217, 219, 220
Rake, G., 57, 231, 232, 260
Rakoto-Ratsimamanga, A., 53
Rammelkamp, C. H., 261, 262, 263
Rankin, L. M., 262
Randall, W. A., 63, 90
Rao, R. R., 206
Raphael, R. A., 183
Rebstock, M. C., 89
Recinos, A., 91
Reed, R., 238
Reedy, R. J., 260
Reese, E. T., 191, 198
Redman, W., 90
Regna, P. P., 199, 246, 273
Reilly, H. C., 220, 221, 231
Reilly, H. P., 85
Reineke, L. M., 94, 230
Reithel, F. J., 180
Renz, J., 126, 265
Reutner, T. F., 90
Reynolds, D. M., 134, 136, 158
Reynolds, H. J., 40
Rice, E. C., 91
Richardson, E. M., 40
Richmond, M. T., 94
Richter, V., 50, 92, 116, 152, 158, 191, 204, 206, 265, 268
Rinderknecht, H., 103, 127
Rittenberg, S. C., 260
Rivett, R. W., 221, 222
Rivière, C., 106
Robbins, W. J., 77, 78, 82, 83, 108, 138, 143, 158, 176, 188, 189, 190
Roberts, E. C., 180

Roberts, J. M., 110
Robertson, A., 100, 265
Robinson, B., 98
Robinson, F. A., 99, 220
Robinson, H. J., 37, 40, 100, 231, 232, 260, 262
Robinson, R., 100, 186
Robson, J. M., 261
Rodaniche, E. C., 261
Rodriquez, J., 65
Roessler, W. G., 231
Rogers, M. M., 199
Rogers, W., 109
Roques, H., 151, 152
Rose, H. M., 246
Rosenman, S. B., 70, 71
Ross, F., 265
Ross, S., 91
Roth, S., 113, 114
Rothhaas, A., 204
Roualt, J. W., 99
Ruiz-Sanchez, F., 91
Ruoff, P. M., 99
Ryan, E. J., 232
Ryan, F. J., 232

Saint-Rat, L., 112, 113, 191
Saito, K., 145, 147
Salle, A. J., 158, 238, 239
Salvin, S. B., 57
Salomon, L., 106
Sanders, A. G., 169, 204
Sanders, M., 64
Sandusky, W. R., 76
Sarles, W. B., 260
Savage, M. C., 68
Saz, A. K., 66
Scaduto, L., 247
Scaparone, M., 64
Schales, O., 180, 259
Schalm, O. W., 262
Schatz, A., 134, 136, 220, 221, 222
Schlingman, A. S., 90, 91
Schlosser, M. E., 198, 199
Schneider, L. K., 232
Schneierson, S. S., 64
Schnitzer, R. J., 80
Schoenbach, E. B., 64, 65, 199, 246, 262, 263
Schoental, R., 213
Scholz, C. R., 132
Schopf, C., 265
Schultz, F. H., Jr., 76
Schwab, J. L., 90
Scott, J. I., 261
Scudi, J. V., 76
Seebeck, E., 46
Seegal, B. C., 49, 206
Seidman, L. R., 263
Selbie, F. R., 261
Sergiev, P. G., 262
Shan, R. C., 100
Shafer, M. V., 78, 79
Sharp, A. E., 68, 91
Sharpe, M. E., 181
Shepherd, R. G., 191, 198, 199
Shimwell, J. L., 158
Short, E. I., 199
Short, W. F., 177, 180
Shultz, S., 272, 273
Siddiqui, S., 46
Silber, R. H., 232
Silverman, M., 261
Simonart, P., 136. 137, 164
Singh, B. N., 99, 267, 268
Singher, H. O., 220, 221
Skell, P., 104
Skrimshire, G. E. H., 180
Slatkin, M. H., 76
Smadel, J. E., 90, 91, 246
Small, L. D., 46
Smith, D. G., 231
Smith, G., 99, 103, 126, 127, 169, 183, 208
Smith, G. N., 90
Smith, L. W., 76
Smith, R. M., 85, 89
Snell, N., 158, 169, 170
Snyder, J. C., 246
Solomons, I. A., 199, 246
Soltys, M. A., 58
Soo-Hoo, G., 80
Spencer, E. L., 100, 103, 104, 118, 121
Spies, H. W., 246
Spink, W. W., 64, 65, 90
Sprenger, R. D., 99
Sprunt, D. H., 64
Srinivasa, R. S., 46
Stanley, A. R., 232
Stanley, N. F., 58, 132
Stansly, P. G., 81, 82, 191, 198, 199
Starbird, E., 63
Stark, T. H., 176
Steenken, W., Jr., 238, 246
Sternberg, H. E., 260
Steude, R., 49
Stieg, W. E., 273
Stodola, F. H., 198, 199
Stokes, J. L., 213, 258, 260
Stokinger, H. E., 263
Stoll, A., 46, 265
Stolovy, E., 232
Strack, E., 213
Streicher, C. J., 262
Strong, F. M., 52, 268

Stubbs, J. J. S., 237
St. Vincent, L., 112
Styles, H., 220, 221
Su, T. L., 160, 161
Suntola, S., 265
Suter, C. M., 46
Suzuki, Y., 99
Swart, E. A., 174
Sweet, L. K., 64
Swift, P. N., 199
Sylva-Goytia, R., 64
Synge, R. L. M., 258, 259
Szobel, D. A., 147

Taira, T., 99
Takman, B., 171
Taliaferro, L. G., 261
Tamiya, H., 99
Tang, P. S., 209
Tauber, H., 99, 234
Teng, P., 76, 77
Tetrault, P. A., 92, 94, 201
Thatcher, F. S., 99
Thayer, J. D., 82, 83, 232, 234
Thayer, S. A., 121
Thaysen, A. C., 162, 163, 164
Thelen, H. M., 64
Thély, M., 106
Theorell, H., 214
Thomas, C. D., Jr., 246
Thompson, B., 260
Thomson, A. M., 104
Thorne, C. B., 270, 271
Tillett, W. S., 247
Timonin, M. I., 99, 100
Timpanelli, A. E., 247
Timreck, A. E., 199
Tishler, M., 40, 232, 258
Tobie, W. C., 57, 58
Todd, A. R., 270
Todd, P., 90
Toharsky, B., 64
Tolmach, J. A., 104
Tompsett, R., 238
Tonkin, I. M., 232
Townley, R. W., 132
Traub, R., 91
Trenner, N. R., 258
Tripp, L. H., 262
Trippet, S., 53
Troutman, H. D., 89
Trussel, P. C., 40, 230, 260
Twible, E., 91

Umezawa, H., 104
Ungar, J., 104
Unwin, C. H., 46, 47
Uzman, L., 259

Vaisman, A., 180
Vallée, M., 239
Van Assendelft, F., 246
Van Bruggen, J. T., 180
Vander Brook, J., 76
Van de Sand, V. F., 188, 204, 267, 268
Vankataraman, P. S., 46
Varella, G., 90
Vekane, K., 104
Venkatamaran, K., 99, 100
Verder, A. E., 199
Villela, G. G., 238
Vonkennel, J., 166

Waksman, S. A., 38, 40, 83, 85, 100, 103, 104, 118, 121, 123, 132, 134, 136, 161, 162, 171, 173, 174, 180, 186, 220, 221, 222, 224, 225, 230, 231
Walker, A. E., 232
Walker, T. K., 157
Wallmark, I. G., 68
Walti, A., 230, 231
Walton, R. B., 169, 170
Ward, A. T., 260, 262
Ward, C. B., 57, 58
Ward, J. L., 103, 127
Warren, G. H., 70, 71
Warth, P. T., 246
Washington, J. A., 91
Waters, R. M., 76
Weerts, E., 107
Wei-Chang, C., 99
Weindling, R., 129, 132
Weinman, D., 260
Weinstein, L., 261, 263
Weinstein, S., 65
Weisenborn, F. L., 104
Weiser, R. S., 112
Welch, H., 63, 247
Weldy, M. L., 261
Wellman, W. E., 247
Wells, I. C., 213
Wells, P. A., 147
Welsch, M., 37, 38, 40, 230, 231
West, H. D., 78
Whalley, W. B., 99
Whiffen, A. J., 35, 37
White, E. C., 53, 57
White, H. J., 191, 198, 199
White, T., 258
Whitehead, H. R., 110, 111
Whitney, D. M., 64
Wien, R., 104
Wiesner, B. P., 100, 103
Wilcox, C., 246
Wiley, J., 76
Wilhelm, S. F., 65

Wilkins, W. H., 100, 103, 118, 121, 128, 143, 145, 147, 158, 160, 169, 241, 242
Wilkinson, S., 198, 199
Will, D., 104
Williams, R. I., 118, 121, 147
Willstaedt, H., 147
Winterbottom, R., 199
Wintermere, D., 90
Wintersteiner, A., 230
Wintersteiner, O., 57, 121, 123, 132
Wolf, L. M., 90
Wolfe, C. K., 246
Wolfson, S. W., 260
Wolinsky, E., 238, 246
Wong, S. C., 64, 238
Wood, R. H., 65
Woodruff, H. B., 38, 40, 69, 169, 170, 183, 217, 220, 225, 230, 231
Woodward, C. R., Jr., 57, 213, 258, 260
Woodward, T. E., 90, 91
Woolfe, G., 104
Worrel, C. S., 90
Wrede, F., 204, 213
Wright, J. M., 46, 47
Wright, L. T., 64
Wyllie, J., 99

Yabuta, T., 145, 146
Yakobson, L. M., 116
Yamatodani, S., 99
Yanagita, T., 99
Yeager, G. H., 246
Youmans, A., 90
Youmans, G. P., 90
Youngs, G., 213
Yow, E. M., 90

Zellat, J. S., 198, 208
Zellert, A. J. S., 99
Zetterberg, B., 147
Zil'ber, L. A., 116
Zittle, P. A., 260
Zweig, J., 213

Index of Organisms

Absidia glauca, 47
Achorion, 166
  A. gypseum, 248, 254
  A. schoenleinii, 170, 248
Achromobacter butylicum, 228
Acetobacter, 145
Acrostalagmus cinnabarinus, 83
Actinobacillus lignieresi, 153
Actinomyces (also see *Streptomyces*), 117, 150, 175, 185, 239, 269
  A. albus, 228
  A. fradii, 214
  A. graminis, 200
  A. israeli, 73
  A. lavendulae, 228
  A. scabies, 168
Actinomycetes, 166, 167
Aerobacter, 245
  A. aerogenes, 39, 42, 56, 60, 73, 79, 84, 87, 93, 102, 120, 131, 135, 141, 150, 172, 178, 179, 195, 197, 206, 209, 214, 216, 222, 227, 228, 234, 240, 243, 244, 271
  A. cloacae, 73
  A. polymyxa, 214, 222
Agaricus (*Clitocybe*) *nebularis*, 171
Alcaligenes, 60
  A. fecalis, 42, 87, 150, 240
  A. metalcaligenes, 87
  A. viscosus, 271
Alectoria, 263
Allescheria boydii, 205
Allium sativum, 43
Alternaria, 163
  A. citri, 157
  A. porri, 46
  A. solani, 46, 51
Amoebae, 179, 245, 267
Anaerobic bacteria, 223
Anemone pratensis, 48
  A. pulsatilla, 48, 205
Anti-coliphage, 179
Ascaris suis, 236
Ascochyta, 51
Ascomycetes, 137
Asparomyces urae, 36
Aspergillaceae, 166
Aspergillus, 166, 184
  A. albus, 145
  A. awamori, 145
  A. candidus, 39, 81, 95, 145
  A. clavatus, 100, 145
  A. effusus, 145, 146
  A. flavus, 53, 55, 56, 145, 146
  A. fumigatus, 81, 118, 121, 129, 145, 195, 214, 217, 218, 248
  A. giganteus, 100, 145
  A. glaucus, 145
  A. gymnosardae, 145
  A. lutescens, 145, 146
  A. luteo-virescens, 145, 146
  A. melleus, 182
  A. nidulans, 145
  A. niger, 39, 45, 49, 58, 131, 134, 157, 160, 163, 170, 214, 228, 269
  A. ochraceus, 182
  A. oryzae, 133, 145, 157
  A. parasiticus, 145, 163
  A. quercinus, 182
  A. sulphureus, 182
  A. tamarii, 145
  A. terreus, 95, 100, 126
  A. ustus, 265, 266
Azotobacter agile, 223
  A. chroococcum, 228
  A. indicum, 228
  A. vinelandii, 39, 229

Bacillus, 175, 223, 272
  B. acoidcae, 168
  B. aerosporus (also see *Bacillus polymyxa*), 191
  B. albolactis, 271
  B. alcaligenes, 73
  B. alvei, 47
  B. anthracis, 42, 48, 67, 69, 73, 79, 87, 93, 105, 110, 120, 122, 124, 127, 141, 150, 153, 157, 161, 172, 183, 188, 203, 205, 208, 209, 212, 216, 219, 233, 236, 240, 267
  B. brevis, 116, 178, 251, 271
  B. cereus, 42, 45, 78, 84, 102, 120, 135, 150, 173, 222, 228, 240, 243, 270
  B. cereus v. *mycoides*, 51, 157
  B. circulans, 92, 240
  B. fasciens, 144

Bacillus — Continued
  B. fusiformis, 222, 271
  B. graveolens, 209, 271
  B. krzemieniewski, 199
  B. laterosporus, 149
  B. licheniformis, 66, 152
  B. megatherium, 84, 102, 120, 135, 222, 228, 240, 267, 271
  B. mesentericus, 42, 84, 131, 151, 240, 267, 271
  B. mycoides, 39, 42, 77, 78, 83, 84, 85, 86, 87, 97, 100, 102, 118, 120, 151, 159, 162, 172, 173, 178, 190, 200, 219, 221, 222, 227, 228, 240, 271
  B. niger, 271
  B. polymyxa, 81, 191, 194, 228
  B. prodigiosus (also see *Serratia marcescens*), 216
  B. simplex, 217
  B. subniger, 271
  B. subtilis, 38, 39, 42, 45, 52, 60, 68, 69, 70, 71, 72, 73, 77, 78, 79, 81, 83, 84, 85, 86, 87, 91, 93, 97, 100, 109, 110, 112, 114, 117, 120, 122, 124, 127, 131, 135, 141, 144, 148, 151, 157, 159, 161, 162, 163, 167, 168, 169, 170, 172, 173, 178, 179, 190, 195, 200, 204, 205, 206, 212, 213, 216, 219, 221, 223, 225, 227, 228, 232, 233, 234, 239, 240, 243, 248, 264, 267, 271, 272
  B. tumefaciens, 144, 168
  B. vulgatus, 271
Bacteriophage, 179
Bacteroides, 245
Bartonella bacilloformis, 255
Basidiomycetes, 108, 137, 138, 143, 176, 188
Blastomyces braziliensis, 71
  B. dermatitidis, 36, 70, 131, 200, 228, 248, 267
Bodenheimer's organism, 173
Boletus bovinus, 131
  B. elegans, 131
Borrelia, 80
  B. novyi, 80, 87
  B. obermeieri, 80
  B. recurrentis, 87
  B. sogdianum, 108
  B. Tick strain, 80
Botrytis allii, 47, 128, 129, 130, 133, 137, 268, 269
  B. cinerea, 157, 163
Brucella, 61, 185, 223, 245
  B. abortus, 39, 42, 81, 87, 93, 105, 110, 131, 151, 153, 172, 178, 179, 195, 213, 214, 228, 229, 234, 240, 267
  B. bronchiseptica, 87, 93, 192, 194, 243
  B. melitensis, 42, 87, 151, 172, 178, 179, 213, 234, 240
  B. suis, 42, 79, 87, 151, 172, 179, 196, 213, 234
Buellia canescens, 11
Byssochlamys fulva, 133

Candida albicans, 36, 49, 70, 173, 205, 248, 254
  C. guilliermondi, 170
Cassia reticulata, 82
Centenella asiatica, 53
Cephalosporium, 166, 269
Ceratostomella paradoxa, 163
Cetraria islandica, 263
Chaetomium bostrychodes, 170
  C. cochliodes, 83
  C. elatum, 83, 133
  C. globosum, 133, 160
  C. spirale, 83
Chlorella pyranoidosa, 91
  C. vulgaris, 91
Cholara quercina, 51
Cholera, 116
Chromobacterium, 92
  C. iodinum, 142
  C. prodigiosum — see *Serratia marcescens*
  C. violaceum, 42, 151, 179, 240, 267
Ciliates, 267
Cladina, 263
Cladonia alpestris, 263
  C. cristatella, 263
  C. sylvatica, 263
Cladosporium herbarum, 269
Claviceps purpurea, 168
Clitocybe gigantea v. *candida*, 105
Clostridium, 87, 175, 245
  C. bifermentans, 56
  C. butyricum, 84
  C. edematiens, 67, 120, 205, 239
  C. fallax, 200, 233
  C. histolyticum, 45, 56, 73, 200, 205, 229, 233, 255
  C. novyi, 56, 67, 73, 205, 227, 233
  C. oedematis-maligni, 233
  C. perfringens, 56, 105, 172, 200, 229, 233
  C. septicum, 56, 67, 73, 120, 175, 227, 244
  C. sordelli, 73, 227
  C. sporogenes, 56, 161, 205
  C. tertium, 200, 233
  C. tetani, 39, 73, 131, 227, 244
  C. welchii, 39, 45, 67, 73, 110, 120, 131, 144, 161, 195, 205, 227, 255, 256, 267
Coccidiodes immitis, 36, 70, 190, 205, 248
Cochliobolus miyabeanus, 51

Coliform bacteria, 229, 243, 244
Colletotrichum circinans, 51
  C. graminicolum, 51
  C. lagenarium, 51
  C. lindemuthianum, 51
  C. lini, 269
  C. phomoides, 51
  C. pisi, 51
Colon-typhoid bacilli, 117, 185, 223
Coniophora cerebella, 137
Corticum solani, 163
Corynebacterium, 165, 175, 223
  C. diphtheriae, 42, 67, 73, 79, 87, 110, 116, 117, 120, 127, 140, 151, 153, 168, 172, 178, 188, 195, 203, 204, 205, 212, 240, 267
  C. diphtheriae gravis, 46, 68, 124, 131, 149, 157, 161, 242
  C. diphtheriae intermedius, 124
  C. diphtheriae mitis, 112, 124, 161, 216
  C. diphtheroides, 87
  C. equi, 153
  C. hofmanni, 203, 205, 212
  C. michiganense, 168
  C. ovis, 153
  C. pyogenes, 153, 195
  C. renale, 153
  C. sepedonicum, 168
  C. xerosis, 42, 48, 67, 69, 71, 73, 107, 149, 151, 161, 168, 187, 205, 212, 213, 236, 240, 242
Crepis taraxacifolia, 108
  C. virens, 108
Crotolaria crispata, 95
Cryptococcus, 117
  C. hominis, 73
  C. neoformans, 36, 170, 205, 228, 248, 272
Ctenomyces radians, 190

Debaryomyces globosum, 36
  D. gruetzii, 170
  D. histolytica, 248
Dermacentroxenus rickettsi, 88, 244
Diatora, 263
Diphtheroids, 73
Diplococcus pneumoniae, 42, 45, 56, 60, 62, 67, 80, 87, 150, 153, 178, 179, 188, 200, 205, 212, 213, 217, 227, 228, 229, 234, 244, 255
  D. pneumoniae I, 69, 240
  D. pneumoniae II, 69
  D. pneumoniae III, 120, 240
Dipodescus uninucleatus, 170

Eberthella muriseptica, 228
  E. typhimurium (also see *Salmonella typhimurium*), 45, 97
  E. typhi (also see *Salmonella typhosa*), 42, 45, 65, 69, 80, 92, 97, 102, 105, 108, 110, 112, 131, 143, 150, 155, 173, 178, 179, 181, 183, 188, 190, 195, 196, 197, 200, 201, 206, 227, 228, 233, 234, 240, 254, 255, 264, 267
Ectothrix, 168
Eleocharis tuberosa, 209
Endamoeba histolytica, 63, 87, 88, 203, 236, 243, 245
Endodermophyton, 168
Endomyces magnusii, 36
Endomycopsis albicans, 133, 242
Endothrix, 168
Enterococcus, 243
Epidermophyton, 131, 166, 168
  E. floccosum, 36, 70, 117, 200, 248, 267, 272
  E. inguinale, 170, 228
Erwinia amylovora, 51
Erysipelothrix monocytogenes, 175
  E. rhusiopathiae, 61, 105, 153, 195, 196
Escherichia, 245
  E. coli, 39, 41, 42, 43, 45, 48, 49, 56, 60, 61, 62, 65, 69, 71, 73, 77, 78, 79, 81, 83, 84, 86, 91, 92, 93, 97, 100, 102, 105, 106, 107, 110, 111, 112, 114, 120, 122, 124, 127, 128, 131, 135, 141, 143 144, 149, 150, 153, 155, 159, 161, 163, 168, 171, 172, 173, 178, 179, 182, 183, 188, 190, 191, 192, 194, 195, 196, 197, 200, 201, 203, 204, 205, 206, 209, 213, 214, 216, 219, 221, 222, 223, 225, 227, 228, 229, 233, 239, 240, 242, 243, 244, 245, 248, 254, 255, 256, 264, 266, 267, 271
  E. coli-commune, 97, 208, 209, 236
  E. coli, streptomycin-resistant, 87, 172, 173
  E. coli, streptomycin-sensitive, 173
  E. communior, 42, 150, 200, 240
  E. intermedia, 60
  E. neapolitana, 240
Evernia, 263

Favus, 168
Ficus carica, 45
Flagellates, 245, 267
Flavobacterium, 73
Flora, intestinal tract, 116, 244, 256
Fomes annosus, 131
Fungi, 87, 146, 157, 168, 173, 179, 185, 203, 223, 236
Fungi-Imperfecti, 137
Fusarium, 39, 148, 166
  F. avenaceum, 148
  F. coeruleum, 47, 133, 134, 269

Fusarium — Continued
  F. culmorum, 269
  F. fructigenum, 148
  F. graminearum, 133, 134
  F. javanicum, 143
  F. lateritium, 147, 162
  F. lycopersici, 157
  F. moniliforme, 170
  F. orthoceras v. *enniatinum*, 113
  F. oxysporum v. *conglutinans*, 51, 248
  F. oxysporum v. *cubense*, 162, 163
  F. oxysporum v. *culmorum*, 163
  F. oxysporum v. *lateritium*, 163
  F. oxysporum v. *lini*, 163
  F. oxysporum v. *lycopersici*, 51, 214, 248
  F. oxysporum v. *nicotianae*, 51
  F. oxysporum v. *pisi*, 51, 248
  F. sambucinum, 148
  F. sciroi, 113

Gaffkya tetragena, 42, 87, 150, 178, 240
Geotrichum, 36
Gibberella saubinetti, 133
  G. zeae, 51
Gliocladium fimbricatum, 129
Gliomastix convoluta, 160
Glomerella cingulata, 51
Gram-negative bacteria, 146, 157, 165, 181, 185, 223, 255
Gram-positive bacteria, 165, 181, 185, 223, 236, 255
Gymnoascus, 100

Haemotomma, 263
Hansenia apiculata, 36
Hansenula anomala, 36, 170
Helminth, intestinal, 245
Helminthosporum avenae, 131
  H. sativum, 51
Hemophilus, 185, 223
  H. bronchisepticus, 195, 196
  H. hemolyticus, 60
  H. influenzae, 60, 62, 67, 173, 195, 196, 204, 228, 244, 257
  H. influenzae B, 73, 131
  H. parainfluenzae, 67
  H. pertussis, 87, 131, 173, 195, 196, 197, 243, 244, 245, 267
  H. suis, 228
Histoplasma capsulatum, 71, 87, 190, 200, 248
Hormodendrum, 117
  H. compactum, 36
  H. pedrosoi, 36, 71
Humicola, 39
Humulus lupulus, 155
Hydnum coralloides, 134
Hydrocotyle asiatica, 53

Inoloma traganum, 141

Klebsiella, 88, 245
  K. pneumoniae, 42, 43, 56, 60, 61, 77, 78, 81, 83, 87, 93, 131, 135, 141, 150, 151, 159, 173, 179, 190, 195, 196, 200, 219, 234, 240, 243, 256, 264
  K. pneumoniae A, 244
  K. pneumoniae R, 173
Kloeckera apiculata, 36

Lactarius deliciosus, 147
Lactobacillus, 175
  L. casei, 131, 228, 234, 236
  L. helveticus, 236
  L. plantarum, 236
Lecanora, 151, 263
  L. esculenta 151
  L. sordida, 216
Leishmania donovani, 203, 236
  L. tropica, 255
Leuconostoc, 168
Lepidium sativa, 151
Leptospira canicola, 146
  L. icterohemorrhagiae, 146, 255
Listerella monocytogenes, 84
Lycopersicon pimpinellifolium, 247

Macrosporium, 51
Malleomyces mallei, 87, 105, 172, 228
Marasmius conigenus, 158
Melanospora destruens, 163
  M. pampeana, 134
Memnionialla echinata, 160
Meningococcus (also see *Neisseria meningitidis*), 195, 255
"Meredith's actinomycete," 162
Metarrhizium glutinosum, 133
Micrococcus, 67, 73, 142, 160
  M. auranticus, 42, 151, 240
  M. conglomeratus, 233, 234, 236, 254
  M. lysodeikticus, 67, 131, 135, 140, 157, 170, 175, 205, 228, 233, 236, 240
  M. flavescens, 271
  M. pyogenes, 245
  M. pyogenes v. *aureus*, 93, 157
  M. roseus, 240
  M. subcitreus, 271
  M. tetragenus (also see *Gaffkya tetragena*), 67, 120
  M. varians, 160
Micromonospora, 161
  M. canis, 205, 228, 272
Microsporon, 166, 168
  M. audouini, 45, 70, 102, 170, 200, 205, 248, 272
  M. gypseum, 70, 117, 254
  M. lanosum, 170, 200

Minor flavus, 170
Monilia, 117
  M. albicans (also see *Endomycopsis albicans*), 73, 102, 195, 200, 272
  M. sitophila, 133
Monosporium apiospermum, 36, 71
Moraxella lacunata, 62
Moringa pterygosperma, 206
Mucor erectus, 133
  M. mucedo, 133, 134
  M. racemosus, 214
  M. stolonifer, 49
Musa sapientum, 162
Mycobacterium, 81, 127, 165, 171, 173, 221, 223, 236, 264
  M. avium, 93, 221
  M. leprae, 228
  M. paratuberculosis, 113, 114, 115
  M. phlei, 47, 48, 52, 67, 68, 71, 77, 78, 83, 84, 87, 97, 107, 114, 124, 135, 144, 148, 149, 152, 153, 157, 163, 172, 187, 190, 206, 216, 219, 228, 236, 264
  M. ranae, 45, 81, 221, 266
  M. smegmatis, 42, 52, 78, 81, 83, 97, 112, 120, 124, 127, 149, 151, 159, 190, 213, 219, 228, 240, 264
  M. synxanthum, 131
  M. tuberculosis, 48, 52, 53, 56, 65, 77, 78, 82, 87, 105, 112, 117, 120, 135, 145, 147, 148, 149, 153, 155, 157, 161, 166, 167, 175, 176, 185, 188, 190, 200, 214, 228, 233, 236, 237, 266, 271
  M. tuberculosis avis, 84, 172, 195, 205, 264, 271
  M. tuberculosis bovis, 42, 52, 105, 151, 195, 205, 216, 264
  M. tuberculosis hominis, 56, 84, 93, 114, 144, 157, 166, 195, 205, 213, 228, 229, 264
  M. tuberculosis R., 172
Mycoderma, 49
  M. valida, 170
Mycorrhizum radicis nigro-strigosum, 131
  M. radicis atrovirens, 131
Mycospaerella citrullina, 51
Mycotorula roseo-corralina, 36
Myrothecium verrucaria, 47, 160

Neisseria, 175, 185
  N. catarrhalis, 42, 60, 79, 87, 93, 131, 151, 178, 188, 195, 212, 236 240, 254, 267
  N. gonorrheae, 60, 62, 67, 73, 83, 195, 233, 236, 255, 257
  N. intracellularis, 62, 67, 120, 131, 172, 227, 267
  N. meningitidis, 60, 73, 87, 140, 203
  N. sicca, 240
Nematospora coryli, 170
  N. phaseoli, 36
Neurospora crassa, 51, 134
Nigrospora sphaerica, 51
Nocardia asteroides, 36, 71, 73
  N. coeliaca, 175
  N. (*Proactinomyces*) *gardneri*, 202

Oidiodendron fuscum, 123
Oidium lactis, 49
Oomycetes, 137

Parmelia, 151, 263
Pasteurella, 67, 69, 105, 233, 239
  P. avicida, 87, 105, 255
  P. boviseptica, 195
  P. bubalseptica, 87
  P. cuniculicida, 87
  P. leptiseptica, 227
  P. multocida, 56, 61, 194, 195, 196, 197
  P. muriseptica, 195
  P. oviseptica, 87
  P. pestis, 67, 172, 195, 233
  P. pseudotuberculosis, 228
  P. septica, 153
  P. tularensis, 87, 172, 196, 244, 255
Penicillium, 39, 100, 124, 129, 181, 184
  P. aurantio-virens, 207, 208
  P. brevi-compactum, 167
  P. chrysogenum, 228
  P. chrzaszszi, 94
  P. cinarescens, 217, 218
  P. citreo-sulfuratum, 94
  P. citrinum, 94, 95
  P. claviforme, 100
  P. corylophylum, 177
  P. cyclopium, 182
  P. cyclopium-viridicatum, 207, 208
  P. daleae, 145
  P. digitatum, 47, 131, 134, 157, 269
  P. divergens, 100, 124
  P. expansum, 94, 100, 133, 134, 269
  P. gladioli, 128, 133, 134
  P. griseofulvum, 136, 164
  P. herquei, 138
  P. implicatum, 94
  P. italicum, 131
  P. janczewski, 136
  P. jenseni, 129
  P. johannioli, 207, 208
  P. leucopus, 100
  P. lividum, 94
  P. notatum, 45, 160, 170, 177, 184, 214, 248, 264, 267, 269
  P. obscurum 129
  P. patulum. 100, 124, 136
  P. phoeniceum, 187
  P. phoeo-janthinellum, 94

Penicillium — Continued
  P. puberulum, 81, 182, 207, 208
  P. resticulosum, 177
  P. rubrum, 187
  P. spinulosum, 217, 218
  P. stoloniferum, 167
  P. suavolens, 182
  P. tardum, 241
  P. thomii, 182
  P. urticae, 100, 124
Phialophora verrucosa, 36, 71, 190
Phoma betae, 133
  P. lingam, 51
  P. radicis callunae, 131
  P. terrestris, 51
Photobacterium fischeri, 56, 77, 97, 159, 190, 219, 255
Phycomyces blakesleeanus, 51, 160
Phytomonas michiganensis, 84, 131
  P. phaseoli, 84
  P. prunei, 84, 182
  P. solanacearum, 248
Phytophthora citrophthora, 157
  P. cryptagea, 133
  P. erythroseptica, 168
  P. palmivora, 133
Pichia membranaefaciens, 36
Pityrosporum ovale, 36
Plasmodium gallinaceum, 229, 256
  P. lophurae, 88
Platysma, 263
Pleuropneumonia-like, 60, 195
Pleurotus griseus, 189
Plumbago europoea, 190
  P. zeylonica, 190
  P. rosea, 190
Pneumococcus (also see *Diplococcus pneumoniae*), 39, 56, 61, 73, 88, 108, 131, 175, 190, 195, 196, 236, 243, 244, 245, 256, 264, 267
Polyporus biformis, 77, 78
Polystictus sanguineus, 201
Proactinomyces cyaneus v. *antibioticus* (also see *Nocardia*), 154
Proteus, 62, 67, 73, 87, 120, 203, 214, 243, 244, 245
  P. morgani, 60, 195
  P. vulgaris, 42, 60, 61, 84, 86, 87, 92, 93, 97, 102, 108, 110, 124, 131, 135, 141, 143, 151, 161, 172, 179, 183, 195, 213, 222, 227, 228, 229, 240, 243, 255. 256, 264, 267, 271
Protozoa, 105, 185, 223
Pseudomonas, 62, 245
  P. aeruginosa, 42, 48, 56, 60, 67, 73, 77, 78, 81, 83, 84, 87, 91, 93, 97, 102, 105, 107, 109, 110, 120, 131, 135, 139, 141, 144, 149, 151, 153, 159, 161, 173, 178, 188, 195, 196, 197, 200, 203, 210, 214, 216, 219, 222, 227, 228, 234, 240, 242, 243, 256, 264
  P. angulata, 214
  P. fluorescens, 84, 127, 131, 135, 222, 228
  P. marginalis, 168
  P. syringae, 168
  P. tobaci, 214
Pythium, 51, 157
  P. graminicolum, 51

Ramalina capitata, 263
  R. reticulata, 263
Ranunculus acer, 48
  R. alpester, 48
  R. arvensis, 48
  R. bulbosus, 48, 205
  R. hirsutus, 205
  R. japonicum, 48
  R. montanus, 48
  R. sceleratus, 48
Raphanus sativus, 215
Rhizobia, 255
Rhizoctonia, 131
  R. crocorum, 168
  R. solani, 130, 157, 168, 217
Rhizopus, 39, 131
  R. javanicus takela, 170
  R. nigricans, 157
  R. stolonifer, 163
Rhodotorula, 272
  R. glutinis, 36
  R. rubra, 170
Rickettsia akari, 88, 244
  R. burneti, 244
  R. mooseri, 88, 244
  R. prowazeki, 244
  R. orientalis, 88
  R. rickettsii, 244
  R. tsutsugamushi, 244
Rickettsiae, 185, 223
  of boutonneuse fever, 61
  of epidemic typhus, 61, 62, 244
  of murine typhus, 61, 87, 244
  of North Queensland tick typhus, 61
  of Q fever, 61, 62, 87
  of rickettsialpox, 61, 87, 244, 245
  of Rocky Mountain spotted fever, 61, 62, 87, 88, 244
  of scrub typhus, 61, 87, 244, 245
  of South African tick-bite fever, 61
  of typhus, 87, 88
Roccella tinctoria, 116

Sabouraudites audouini, 242
  S. lanosus, 242
  S. schenki, 228

Saccharomyces carlsbergensis, 36, 170
S. cerevisiae, 36, 49, 134, 137, 160, 205, 267
S. ellipsoideus v. *burgundy*, 36
S. fragilis, 36
S. lactis, 36
S. pastorianus, 36, 37
Salmonella, 60, 84, 93, 102, 120, 179, 195, 245
S. abortivoequina, 228
S. aertrycke, 227, 228, 264
S. cholerae-suis, 60, 102, 120, 179, 195
S. derby, 60
S. enteriditis, 43, 56, 67, 69, 87, 93, 97, 107, 124, 131, 141, 149, 151, 179, 183, 195, 203, 206, 213, 227, 234, 240, 242
S. gaertneri, 102, 120, 239
S. gallinarum, 61, 87, 93, 271
S. manhattan, 60
S. newport, 62, 107
S. oranienburg, 214
S. oregon, 60
S. paratyphi, 43, 45, 69, 87, 93, 97, 102, 105, 107, 110, 124, 131, 151, 153, 155, 179, 183, 200, 201, 203, 206, 234, 240, 243, 254, 255
S. paratyphi A, 79, 195
S. paratyphi B, 79, 108, 195
S. pullorum, 60, 93, 135, 173, 195, 228, 243
S. schottmuelleri, 43, 69, 80, 87, 93, 102, 105, 120, 131, 135, 151, 155, 173, 179, 195, 200, 201, 216, 227, 228, 233, 240, 243, 254, 255, 267
S. suipestifer, 62
S. typhimurium, 60, 61, 93, 102, 107, 122, 124, 131, 183, 195, 196, 200, 208, 209, 219, 228, 234, 264
S. typhosa, 60, 61, 67, 73, 79, 87, 92, 93, 102, 107, 109, 120, 141, 149, 161, 168, 173, 194, 203, 216, 243
Sarcina aurantiaca, 87
S. lutea, 39, 42, 45, 79, 81, 84, 85, 86, 87, 100, 102, 118, 120, 122, 131, 135, 141, 151, 157, 161, 162, 173, 178, 227, 228, 236, 240, 271
Schizosaccharomyces pombe, 36
Schwanniomyces occidentalis, 36, 170
Sclerotinia fructicola, 51, 157, 170
S. fructigena, 163
S. laxa, 51
S. minor, 51
S. sclerotiorum, 51
Sclerotium bataticola, 157
Serratia marcescens, 42, 60, 84, 87, 93, 135, 141, 151, 173, 204, 213, 228, 229, 234, 240, 267, 271
Shigella, 88, 245, 264
S. alkalescens, 73, 102, 135
S. dysenteriae, 43, 45, 87, 93, 97, 120, 127, 135, 139, 151, 153, 155, 183, 195, 200, 203, 216, 227, 228, 240, 255
S. flexneri (also see *Shigella paradysenteriae*), 107, 124, 131
S. gallinarum, 194, 195, 197, 228
S. madampensis, 87
S. paradysenteriae, 43, 65, 86, 97, 120, 135, 151, 155, 173, 178, 188, 195, 201, 206, 228, 240, 243
S. schmitz, 195
S. shigae, 107, 108, 124
S. sonnei, 86, 87, 107, 120, 124, 178, 195, 227
Soil bacteria, 179
Spirochaetes, 185
Sporobolomyces roseus, 170
S. salmonicolor, 36
Sporotrichum, 117
S. schenkii, 36, 71, 200, 272
Squamaria, 263
Stachobotrys atra, 47, 134, 269
Staphylococcus, 105, 116, 120, 161, 190, 191, 223, 244, 245
S. albus, 45, 60, 67, 69, 87, 97, 120, 122, 124, 127, 131, 139, 161, 165, 178, 183, 205, 214, 219, 233, 243, 254, 256, 267, 271
S. aureus, 38, 39, 41, 42, 45, 47, 48, 49, 56, 58, 60, 62, 67, 68, 69, 71, 73, 77, 78, 79, 81, 83, 84, 85, 87, 91, 92, 97, 102, 105, 107, 108, 109, 110, 111, 112, 114, 117, 120, 122, 123, 124, 125, 126, 127, 128, 130, 131, 135, 139, 140, 141, 143, 144, 145, 146, 148, 149, 151, 154, 155, 157, 159, 160, 161, 162, 163, 165, 168, 173, 177, 178, 179, 182, 183, 185, 187, 188, 189, 190, 195, 196, 200, 201, 203, 204, 205, 206, 208, 209, 212, 214, 215, 216, 217, 218, 219, 221, 227, 228, 229, 233, 234, 236, 239, 240, 241, 242, 243, 244, 248, 254, 255, 256, 264, 267, 268, 270, 271
S. aureus, penicillin-resistant, 213
S. citreus, 60, 87, 97, 120, 124, 127, 183, 208, 209
S. coagulase positive, 73, 244
S. pyogenes, 109, 140, 141, 144, 148, 149, 151, 153, 163, 165, 168, 175, 188, 195, 196, 200, 201, 203, 204, 208, 209, 233, 236, 237, 240, 264
Stemphylium, 134, 269
S. consortiale, 160
S. sarcinaeforme, 51
Stereum hirsutum, 139
S. purpureum, 134, 168

Streptococcus, 39, 60, 69, 88, 105, 110, 116, 120, 153, 161, 175, 195, 214, 223, 233, 244, 245, 255, 256
  S. agalactiae, 74, 174, 195, 256
  S. alpha, 43, 56, 151, 240
  S. cremoris, 111
  S. fecalis, 45, 56, 60, 62, 67, 93, 110, 157, 161, 173, 195, 236, 240, 244, 254, 255, 256
  S. gamma, 43, 151, 240
  S. hemolyticus, 56, 60, 61, 65, 72, 73, 81, 87, 92, 97, 111, 120, 143, 144, 155, 178, 179, 190, 205, 212, 213, 227, 228, 244, 254, 255, 256, 257, 264, 267
  S. infrequens, 87
  S. lactis, 87, 105, 111, 131, 227, 254
  S. mitis, 60
  S. non-hemolytic, 73, 87, 244, 267
  S. pneumoniae (also see *Diplococcus pneumoniae*), 175, 195, 203
  S. pyogenes, 42, 48, 56, 60, 67, 91, 102, 105, 109, 110, 120, 124, 127, 131, 139
  S. salivarius, 65, 188, 256
  S. uberis, 256
  S. viridans, 67, 97, 110, 120, 122, 127, 131, 153, 155, 165, 183, 195, 196, 201, 203, 205, 208, 209, 212, 227, 242, 254, 267
Streptomyces, 38, 41, 50, 52, 112, 220, 221, 225, 270
  S. albus, 37
  S. antibioticus, 38
  S. aureofaciens, 58
  S. coelicolor, 157
  S. fradiae, 171
  S. griseus, 35, 81, 134, 220, 222
  S. lavendulae, 150, 179, 225
  S. rimosus, 242
  S. rochei, 79
  S. venezuelae, 85
  S. violaceus, 37, 165
Syncephalastrum racemosum, 134, 195

Tetrahymena geleii, 205
Thamnidium elegans, 133, 134
Tilachlidium, 110
Tilletia caries, 131
Torula cremoris, 170
  T. histolytica, 71
  T. utilis, 36
Torulaspora fermentati, 36
Torulopsis delbruckii, 170
  T. utilis, 134, 137
Treponema, 185
  T. pallidum, 73, 74, 88, 236, 237
Trichoderma, 39, 131, 170
  T. lignorum, 129
  T. viride, 129, 134, 268, 269
Trichomonas foetus, 221
  T. gallinae, 221
  T. vaginalis, 220, 221, 255
Trichophyton, 169, 170
  T. equinus, 242
  T. ferrugineum, 190
  T. gypseum, 45, 102, 228, 248, 254, 272
  T. lacticolor, 242
  T. interdigitale, 43, 151, 179, 228, 240, 248
  T. mentagrophytes, 36, 70, 117, 160, 170, 173, 195, 248
  T. purpureum, 102
  T. rubrum, 36, 70, 200, 248, 267, 272
  T. sabouraudii, 242
  T. schoenleini, 70
  T. tonsurans, 242
Trichothecium roseum, 134, 249, 269
Trypanosoma cruzi, 255
  T. equiperdum, 39, 203, 236, 237
  T. gambiense, 205
  T. lewisi, 255
Typhus bacteria, 116

Usnea barbata, 263
  U. dasypoga, 263
  U. florida, 263
  U. hirta, 263
Ustilago hordei, 131
  U. zeae, 170

Venturia inaequalis, 51
Verticillium cinnabarinum, 83, 134
  V. dahliae, 134, 163, 168
Vibrio comma, 42, 67, 87, 93, 97, 107, 122, 124, 127, 139, 151, 153, 155, 161, 173, 179, 183, 195, 201, 203, 208, 209, 212, 219, 239, 240, 267
Virus, 185, 223
  of distemper, 88
  of epidemic influenza, 229
  of feline pneumonitis, 229
  of foot-and-mouth disease, 105
  of fowl pox, 39
  of granuloma inguinale, 62
  of herpes, 62, 244
  of influenza, 88, 205, 243, 244, 245
  intestinal, 88
  of Japanese encephalitis, 88
  of laryngotracheitis, 39
  of lymphogranuloma venereum, 62, 87, 229
  of mumps, 88
  of Newcastle disease, 88

of poliomyelitis, 88
of psittacosis, 61, 87, 88, 244
of psittacosis-lymphogranuloma group, 61
of rabies, 88, 244
of Rous sarcoma, 179
of St. Louis encephalitis, 88
of vaccinia, 88
of variola, 88

Xanthomonas begoniae, 168
  X. campestris, 168
  X. malcacearum, 168
  X. translucens, 237

Yeast, 87, 131, 146, 157, 185, 213, 223, 229

Zygomycetes, 137
Zygopichia californica, 170

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